

ANNALS OF THE NEW YORK ACADEMY OF SCIENCES
VOLUME 57, ART. 6 PAGES 615-962

Editor
ROY WALDO MINER

NUTRITIONAL FACTORS AND LIVER DISEASES

BY

KLAUS SCHWARZ (*Conference Chairman*) C H BEST M BEVANS J M R BEVERIDGE,
[F ... D H ... AND W F ... F S ... C S D ...]

Consulting Editor
KLAUS SCHWARZ



NEW YORK
PUBLISHED BY THE ACADEMY
May 10 1954

THE NEW YORK ACADEMY OF SCIENCES
(Founded in 1817)

COUNCIL, 1954

President

JOHN TEE-VAN

President-Elect

MAURICE L. TAINTER

Vice-President

ELMORE H. NORTHEY

Recording Secretary
ROSS F. NIGRELLI

Treasurer

DONALD M. BENJAMIN

Corresponding Secretary
JUNIUS BIRD

Editor

ROY WALDO MINER

Elected Councilors
1952-1954

WILLIAM H. COLE

BORIS PREGEL

JOHN M. CONVERSE

RANDOLPH T. MAJOR

1953-1955
EDWARD J. KEMPF

1954-1956

CHARLES H. TOW

JOHN TURKEVICH

D. M. DUGGA

ABRAHAM SLAVIN

Finance Committee

GORDON Y. BILLARD

HARDEN T. TAYLOR, *Chairman*

ROBERT F. LIGHT

Executive Director

EUNICE THOMAS MINER

SECTION OF GEOLOGY AND MINERALOGY
ANGELINA ROSE MESSINA, *Secretary*

THOMAS N. WALTHER, *Chairman*

SECTION OF BIOLOGY

M. J. KOPAC, *Chairman*

DIVISION OF MYCOLOGY

HILARY KOPROWSKI, *Secretary*

SAMUEL M. PECK, *Chairman*

SECTION OF PSYCHOLOGY

LINDSAY S. OLIVE, *Secretary*

NEWMAN L. HOOPINGARNER, *Chairman*

SECTION OF ANTHROPOLOGY

WILLIAM L. THOMAS, JR., *Chairman*

SECTION OF PHYSICS AND CHEMISTRY

JOHN L. LANDGRAF, *Secretary*

CECIL V. KING, *Chairman*

SECTION OF OCEANOGRAPHY AND METEOROLOGY

J. J. DENTON, *Secretary*

ERNEST J. CHRISTIE, *Chairman*

SECTION OF MATHEMATICS AND ENGINEERING

MAYNARD E. SMITH, *Secretary*

SEBASTIAN B. LITTAUER, *Chairman*

DAVID B. HERTZ, *Secretary*

These Sections and the Division hold meetings regularly, one evening each month, during the academic year October to May inclusive. All meetings are held at the building of The New York Academy of Sciences 2 East Sixty third Street, New York 21, New York

ANNALS OF THE NEW YORK ACADEMY OF SCIENCES

VOLUME 57 ART 6 PAGES 615-962

May 10 1954

Editor

ROY WALDO MINER

NUTRITIONAL FACTORS AND LIVER DISEASES†

Consulting Editor: *Uli Coferre, c. C. Arna* KLAUS S. ARZ

CONTENTS

In du n i e Ne on su Fa l an C rro By k w	61
I a t I F a t y L i e r a l C r e l	
Exner me a D i f f e n a n n L e \ an L e C h o s an b m D a F a o s A f f n g Th De n n By F o n S I a r	6
The S q u e n e n P a h o g E n n h D o n f i p e n n a G a a d C r r h o u B W S L E H R R T	633
The L i o F a o s By C H B E T C C L C A S A V J H R D	646
L p p f f of V a m n B and O b e F a By V C T R A D r L	4
L C a n o m a and R e L e n n C h o n C h o De n B D S m o v n D H C P E L D	4
H e p a o m P o d u n M e b I g B n n e n I B f V r e V O N	68
The L i f of l u r e o m n A d m n a n u n h l o o l D h L a d P a n a D u By A F C K P L A N C A P E N E C O N R n D K E R E J O N E S	89
S u y o h W o d S u a o n o n K u a h o l o I J F B r a	696
T h P a h o g o D a y L D s e a e n T o p a V B J N F D	714
C n a A p s o b e T e a m n o K u a a h l o By H C T r	7
V m n E a n d C a o n o d s n h e B o d P a m n k a h k B H I e T M O O R E A D I M S R M A N	74
A of N u o n a I e D s e a e H u m a n a d f n n J e M A N D C R T F G B E R T	37
F n e A y n F a L n H n a n I n a n s B J C A R O J P R K	5
The Q u o n of h e R e a e I m p a n e of P o n a n L a M h h D o p m n o F a L a e a n l C h o s n M a n B B r M N o	64
R e l a e E f f o P o e n n d L o p S u a n h T e n N u n a C h o w n i a B A r t R J P E K J R I M R G R E R E A S	7

The B p p e r s Th w k A d m S n u n A r y D
P h y Th d e d b g A d m G r y h
R e s r e l

Protein Metabolism in Patients with Cirrhosis of the Liver	By GEORGE J GABUZDA JR AND CHARLES S DAVIDSON	776
Treatment of Infantile Cirrhosis of the Liver with Antibiotics	By P KRISHNA RAO	786
Metabolic and Nutritional Patterns in Alcoholism	By JORGE MARDONES	788
The Genetotrophic Concept—Nutritional Deficiencies and Alcoholism	By ROGER J WILLIAMS	794
Nutritional Aspects of Cirrhosis in Alcoholism—Effect of Purified Diet Supplemented with Choline	By GERALD B PHILLIPS AND CHARLES S DAVIDSON	812

Part II Liver Necrosis

• The Pathology of Dietary Liver Necrosis—A Preliminary Report	By GEORGE L FITE	831
Dietary Methods for Induction of Necrotic Liver Degeneration	By MARIANNE GOETSCH	839
Dietary Hepatic Necrosis in the Rat—Absence of Cirrhosis Following Recurrent Episodes	By F W HOFFBAUER AND BERNADINE WITTENBURG	843
The Effect of Environment and Mode of Feeding and of Rearing on the Production of Acute Dietary Liver Necrosis in the Rat	By J M NATTALEN	862
Effect of Different Makes of Casein on the Production of Acute Dietary Liver Necrosis in the Rat	By J M NATTALEN	869
The Influence of the Endocrine Glands on the Development of Acute Massive Liver Necrosis	By J M R BEVERIDGE	873
• Factors Protecting against Dietary Necrotic Liver Degeneration	By KLAUS SCHWARZ	878
Enzyme Abnormalities Associated with Dietary Necrotic Liver Degeneration in Rats	By ROBERT E OLSON AND JAMES S DUNN	889
The Xanthine Oxidase Factor (Molybdenum)	By W W WESTERFELD AND DAN A RICHERT	896
Studies on the Nature of the Xanthine Oxidase Factor	By EDWARD C DE JENZO	905
The Effects of Liver Disease on Certain Aspects of Tocopherol Metabolism in Man	By GERALD KLATSKIN	909
Phospholipide Synthesis in Liver Metabolism	By W E CORNATZER	919
Antibiotics and Liver Injury	By PAUL GEORGY	925
Germfree Animals and Liver Necrosis	By T D LUCKEY J A REINHARTS PALL GEORGY AND M FORBES	932
Hepatic Injury Due to Conlitioned Sulfo-Amino Acid Deficiency	By HANS POPPER J DE LA HUEGA AND DIETER KOCH WESPE	936
Clinical Evaluation of a High Protein High Carbohydrate Restricted Fat Diet in the Treatment of Viral Hepatitis	By N C LEONF FRANK RATNER WILLIAM C RAY	946

INTRODUCTION: LIVER NECROSIS VERSUS FATTY LIVER AND CIRRHOSIS

By Klaus Schwarz
Laboratory of Biochemistry and Nutrition, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Md

Part of our knowledge we obtain direct and part by argument.
—JOHN MAYNARD KEYNES, *A Treatise on Probability*

It is, of course, a great pleasure to write the introduction to this monograph based on the Conference on "Nutritional Factors and Liver Diseases," which it has been my privilege to plan and to organize. The concepts and intentions which have guided this endeavor can be outlined as follows. Within the limitations of a two-day meeting and the following collection of papers presented, an attempt has been made to assemble a very broad spectrum of our present knowledge and of all current trends in research on nutritional liver disease, to put before you a real "symposium." What appears more important, however, is that an effort has been made to put our various pieces of knowledge together in such a way as to form a clearer picture out of the rather chaotic development which this field has undergone. This may permit us to visualize and to understand possible relations between heretofore isolated areas, to clarify, at least in part, some of the complex aspects of this field, which seemingly have been lacking integration so far.

To achieve this purpose, several heterogeneous fields had to be brought together and connected in a coordinated fashion, the two most important ones being biochemical research in experimental animals on one side and clinical and pathological research on humans suffering from dietary liver disease on the other. Workers not only from various scientific fields but also from different continents had to be brought together since the investigation of experimental dietary liver diseases has advanced most in North America and in Europe, whereas work in human nutritional liver injury has by necessity been carried out more intensely in the arrangement of the following material is where Cecil Williams made her classical observations on kwashiorkor in 1933.¹ Our appreciation is extended to all the workers from abroad who have contributed so effectively to the meeting and to this volume.

The guiding consideration in the arrangement of the following material is based on the results of animal experimentation. It is quite certain that, in experimental animals, two completely different deficiency syndromes can be distinguished clearly. There are fatty liver and cirrhosis on the one hand, and acute (massive) liver necrosis preferably called necrotic liver degeneration,* on the other. These two disease entities can be produced separately and even occupy a mutually antagonistic position in response to various dietary factors which are protective (see below). According to this clear-cut separation, the conference program and this monograph are separated into two main

* Degeneration is defined as progressive deterioration of tissue in which its vitality is diminished whereas necrosis means death of a circumscribed portion of tissue. Dietary necrotic liver degeneration is applied to define a deficiency disease which in previous publications has been called hemorrhagic hemorrhage and acute extensive liver necrosis and death. The defect is characterized by a degenerative change which eventually leads to

parts, one being devoted to fatty liver and cirrhotic diseases and the other to necrotic liver injuries. Each main part, in turn, was scheduled originally to present first the results of animal experimentation and, secondly, the clinical investigations of corresponding nutritional liver injuries in the human. A certain imbalance is evident, however, since experience with human fatty liver and cirrhosis due to malnutrition is abundant indeed, whereas knowledge of human necrotic liver disease due to malnutrition is quite inadequate as yet.

Nutritional Liver Disease in Humans (Kwashiorkor)

It has become quite clear that nutritional disease of the liver occurs very frequently in small children and also in adults, especially in tropical and in subtropical areas, as part of the so called kwashiorkor syndrome. The disease constitutes a world wide problem. It is one of the most important dietary deficiencies in mankind today. This is not yet sufficiently realized in some countries, partly due to indifference and partly due to lack of information and of communication. It is often fatal and poses difficult and challenging problems in the field of clinical medicine and in nutrition research. The disease is not really new. It has been described under a great variety of names for example as kwashiorkor², culebrilla syndrome polarental, malignant malnutrition, dystrophie de farineuse, and as far back as 1906 as Mehlinahrscha den³. The African term kwashiorkor has been officially accepted by the Joint Expert Committee on Nutrition of the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) of the United Nations.

In the liver, the major features of kwashiorkor are fatty infiltration and cirrhosis. These changes are apparently intimately related, if not identical, to fatty infiltration and cirrhosis in areas with temperate climates, particularly among alcoholics. For this reason, the nutritional theories of the etiology of alcoholism, as well as the dietary aspects of the origin and the treatment of alcoholic fatty liver and cirrhosis have been considered as pertaining to the general theme of this volume. The dietary factors which protect against human fatty liver and cirrhosis have not been elucidated as yet.

Experimental Nutritional Liver Disease

In experimental animals, dietary liver diseases (i.e., liver injuries produced

describing "hemorrhages throughout the liver" and "clear signs of icterus" which were produced by a cystine deficient diet was not realized until much later. When du Vigneaud and his associates produced liver necrosis in rats on amino acid diets in 1939 they devoted only one paragraph in small print to the description of the phenomenon⁷. Between 1939 and 1946 difficulties were encountered in the differentiation of these two types of liver diseases, and the resolution of these difficulties was seriously delayed by the international scientific blackout during World War II.

In doing experimental research with purified diets, we have, allegedly, the

advantage of being able to deal with "pure" forms, with just one variable at a time, whereas the clinician deals with more complex situations. However, it is characteristic for both experimental fatty liver/cirrhosis and liver necrosis, that they are the result of rather complicated dietary deficiency situations. Both of these injuries are not the result of single dietary defects, but are caused by the simultaneous lack of several essential nutrients. In both diseases some of the major dietary factors have been identified, but hitherto unknown protective factors are also involved. Hence, it is understandable that research workers have not always succeeded in producing pure forms of these diseases. In 1939, papers appeared describing the occurrence of both liver necrosis and fatty infiltration/cirrhosis in the same animals. These had been kept for extended periods on low protein rations which were deficient in quite a variety of essential dietary components. Thus both types of nutritional liver disease were produced simultaneously, leading some workers to think that those lesions constituted a single deficiency syndrome. This somewhat handicapped progress in the clarification of the factors involved. In 1942 it was clearly established by Daft, Sebrell, and Lillie³ that the two diseases were separate entities. This, however, did not prevent the subsequent publication of occasional papers which failed to discriminate between the fatty liver/cirrhosis syndrome and dietary necrotic liver degeneration. In Europe, during the same period, several dietary methods were found which produced necrotic liver degeneration at high incidence and in a reproducible manner.^{4, 5, 6} Signs of fatty infiltration/cirrhosis were not seen since the rations were supplemented with choline. The necrotic deficiency disease was found to be prevented by cystine⁷ and in 1944 vitamin E was disclosed to be highly protective.⁸ More recently a third, not yet identified factor, Factor 3, has been shown to be involved.⁹ Each one of these three substances alone affords protection

TABLE I

Disease process	LIVER NECROSIS	FATTY LIVER CIRRHOSIS
	degenerative metabolic change of the liver parenchyma leading to sudden, acute attack of massive necrosis	fatty metamorphosis of liver with slowly developing fibrosis/cirrhosis
<i>Residual signs</i>	No fatty infiltration	No (massive) necrosis/cirrhosis
<i>Protective factors</i>	No fibrosis	
Cystine ^a	postnecrotic scarring	
Vitamin F	protects	enhances
Factor 3	protects	without effect
Choline	enhances	not known
Betaine	enhances	protects
Methyl group precursors	enhances	protects
Vitamin B ₁₂	enhances	protects
Folic acid, citrovorum factor	enhances	protects

^a Methionine has been shown to be protective against fatty liver and cirrhosis. It contains a labile methyl group. It is also slightly protective in dietary necrotic liver degeneration since it is partly transformed into cystine in intermediary metabolism.

Thus it became obvious that the group of dietary factors which prevents necrotic liver degeneration is completely different from the group of factors which protects against fatty liver/cirrhosis. Furthermore it could be shown that substances which prevent the development of fatty liver and cirrhosis such as choline, betaine, precursors of one carbon units, vitamin B₁₂ and folic acid/citrovorum factor enhance the development of the necrotic liver injury.¹⁴ On the other hand, cystine, which protects against necrotic degeneration, accentuates the fatty liver/cirrhosis syndrome. The situation is summarized in TABLE I.

*Regarding the Relationship of Experimental and Human
Nutritional Liver Disease*

It is evident from this monograph that the various phases of experimental and of human nutritional liver disease can be put together in such a way that a great number of parallels become visible. This is particularly true for the fatty liver/cirrhosis diseases. However, there are also obvious discrepancies. As to necrotic liver injury, the situation is much less clear, the occurrence of nutritionally induced necrotic disorders in the human remains to be established. I want to state explicitly that the obvious parallelisms do not necessarily

clarified. Knowledge of human nutritional disorders of the liver, by comparison, is much less advanced. The more or less close parallelisms between human liver disease and the two main types of experimental dietary liver injury have of course led to conjecture and to premature discussion of the question of how much human liver disease is induced nutritionally and what might be done with liver protecting factors in therapy. As long as we do not know all the dietary factors necessary to protect, these questions cannot be answered. Early hopes, for example, that choline, which is protective against fatty liver and cirrhosis in the rat, would prevent or cure fatty liver and cirrhosis in the

Between different species or even between different strains of the same kind, differences in the relative importance of these various essentials are naturally bound to occur, and we should not expect the human to respond in all details exactly as the rat does.

The conclusion which can be drawn from this example is that much more careful studies are necessary before the true relationship between experimental and human dietary liver injury can be ascertained. Such relationship is bound to exist, but at present we can not recognize it precisely. It is hoped that a full exchange of knowledge and of opinions—often controversial—will be helpful to both the laboratory investigator and the clinician. This exchange is what has been attempted here.

Among biochemists, pathologists, and doctors there are differences of approach and of thinking with resulting possibilities of misunderstanding. There are also pitfalls in terminology and in semantics. Bringing these groups together constituted something of an experiment in itself, one which appeared well worth trying.

References

- 1 WILLIAMS C D 1933 Arch Disease Childhood 8 473
- 2 BROOK J F & M ATTREY 1952 Kwashiorkor in Africa WHO Monograph Series No 8 Geneva
- 3 CILANY A. & A KELLER 1906 Des Kindes Ernährung Ernährungsstörungen und Ernährungstherapie Leipzig
- 4 BEST C H & J H RIDOUT 1939 Ann Rev Biochem 8 349
- 5 BEST C H & C C LUCAS 1943 Vitamins and Hormones 1 1
- 6 WEICHELBAUM T E. 1935 Quart. J Exptl Physiol 25 363
- 7 DU VIGNEAUD V H M DYER & W W KIES 1939 J Biol Chem 130 395
- 8 DAFT F S, W H SEBRELL & R D LILLIE 1942 Federation Proc. 1 183, 1942
- 9 HOCK A & H FIDAL 1944 Z physiol Chem 278 136, 1943 Ibid 279 187
- 10 SCHWARZ K 1944 Z. physiol Chem 281 101
- 11 GLYNN L E & H P HIMS WORTH 1944 J Path Bact 56 237
- 12 SCHWARZ K 1944 Z. physiol Chem 281 109
- 13 SCHWARZ K 1951 Proc Soc. Exptl Biol Med 78 852
- 14 SCHWARZ K 1952 Federation Proc 11 435

thus it became obvious that the group of dietary factors which prevents necrotic liver degeneration is completely different from the group of factors which protects against fatty liver/cirrhosis. Furthermore it could be shown that substances which prevent the development of fatty liver and cirrhosis, such as choline, betaine, precursors of one carbon units, vitamin B₁₂ and folic acid/citrovorum factor enhance the development of the necrotic liver injury. On the other hand, cystine, which protects against necrotic degeneration, accentuates the fatty liver/cirrhosis syndrome. The situation is summarized

TABLE I

*Regarding the Relationship of Experimental and Human
Nutritional Liver Disease*

It is evident from this monograph that the various phases of experimental and of human nutritional liver disease can be put together in such a way that a great number of parallels become visible. This is particularly true for the fatty liver/cirrhosis diseases. However, there are also obvious discrepancies. As to necrotic liver injury, the situation is much less clear, the occurrence of nutritionally induced necrotic disorders in the human remains to be established. I want to state explicitly that the obvious parallelisms do not necessarily indicate true identity of biochemical and pathological events involved. Much has been achieved in experimental nutritional liver disease even though there are still many important questions and problems which have not been solved. Knowledge of human nutritional disorders of the liver, by comparison, is much less advanced. The more or less close parallelisms between human liver disease and the two main types of experimental dietary liver injury have of course led to conjecture and to premature discussion of the question how much human liver disease is induced nutritionally and what might be done with liver protecting factors in therapy. As long as we do not know the dietary factors necessary to protect, these questions cannot be answered. Early hopes, for example, that choline, which is protective against fatty liver and cirrhosis in the rat, would prevent or cure fatty liver and cirrhosis in the human have not been fulfilled. This has led some to discredit the nutritional approach. Since then we have learned that numerous factors other

differences in the relative importance of these various essentials are naturally found to occur, and we should not expect the human to respond in all details exactly as the rat does.

The conclusion which can be drawn from this example is that much more careful studies are necessary before the true relationship between experimental and human dietary liver injury can be ascertained. Such relationship is hard to exist, but at present we can not recognize it precisely. It is hoped that a full exchange of knowledge and of opinions—often controversial—will be helpful to both the laboratory investigator and the clinician. This exchange is what has been attempted here.

Among biochemists, pathologists and doctors there are differences of approach and of thinking with resulting possibilities of misunderstanding. There are also pitfalls in terminology and in semantics. Bringing these groups together constituted something of an experiment in itself, one which appeared well worth trying.

References

- 1 WILLIAMS C D 1933 Arch Disease Childhood 8 423
- 2 BROOK J F & M AUTRET 1952 Kwashiorkor in Africa WHO Monograph Series No 8 Geneva
- 3 CZERNY A & A KELLER 1906 Des Kindes Ernährung Ernährungsstörungen und Ernährungstherapie Leipzig
- 4 BEST C H & J H RIMOUT 1939 Ann Rev Biochem 8 349
- 5 BEST C H & C C LUCAS 1943 Vitamins and Hormones 1 1
- 6 WEICHELBAUM T E 1935 Quart J Exptl Physiol 25 363
- 7 DU VIGNEAUD V H M DIER & R. D LILLIE 1939 J Biol Chem 130 323
- 8 DART F S W H SEBRELL & R. D LILLIE 1942 Federation Proc. 1 183 1942
- 9 HOCK A & H FINE 1943 Z. physiol Chem 278 136 1943 Ibid 279 147
- 10 SCHWARTZ K 1944 Z. physiol Chem 281 101
- 11 GLYNN L E & H P HINSWORTH 1944 J Path Bact 66 237
- 12 SCHWARTZ K 1944 Z. physiol Chem 281 109
- 13 SCHWARTZ K 1951 Proc Soc. Exptl Biol Med 78 852
- 14 SCHWARTZ K 1952 Federation Proc 11 455

Part I. Fatty Liver and Cirrhosis

EXPERIMENTAL DIFFERENTIATION BETWEEN LIVER NECROSIS AND LIVER CIRRHOSIS AND SOME DIETARY FACTORS AFFECTING THEIR DEVELOPMENT

By Floyd S. Daft

National Institute of Arthritis and Metabolic Diseases, National Institutes
of Health, Bethesda, Md

It is now generally recognized that dietary liver cirrhosis and dietary liver necrosis are separate and distinct entities, both etiologically and morphologically. That is not to say that death of cells does not occur as part of the cirrhotic process or that fibrosis may not follow necrosis. Most observers of experimental dietary cirrhosis have reported the presence of scattered necrotic liver cells. I believe that none would contend, however, that massive necrosis is part of this process. Extensive scarring following massive necrosis has been observed also in several laboratories. We at the National Institutes of Health, however, have never observed diffuse fibrosis following dietary necrosis and the same appears to be true of most other groups of investigators. Doctor Hoffbauer will discuss this matter at some length in a later paper. It suffices to say here that there are two distinct processes, experimental dietary cirrhosis and experimental dietary necrosis, which may occur either separately or in the same liver in rats which have received certain deficient diets.

At this time I should like to review briefly the historical background of this concept and present some additional data in its support. In addition, I should like to discuss a few experiments carried out in our laboratory on the influence of dietary ingredients other than those previously implicated, on the development particularly, of fatty livers and etiologically related lesions.

The first clear differentiation between dietary liver cirrhosis and dietary liver necrosis was made in 1942 by Daft, Sebrell, and Lillie.¹ In our first publication on this subject, in *Federation Proceedings*,¹ we stated, "Under the conditions of our experiments choline prevents the cirrhosis and the hemorrhagic necrosis, and methionine prevents both the cirrhosis and the hemorrhagic necrosis. It appears, therefore, that the cirrhosis and the hemorrhagic necrosis are separate and distinct pathological entities." In our more complete report published a few months later in the *Proceedings of the Society for Experimental Biology and Medicine*² our conclusions were worded somewhat more conservatively, but the reasons for our belief that we were dealing with two distinct deficiency syndromes were clearly stated. The evidence on which our conclusions were based consisted of morphological observations and the results of dietary experiments. In regard to the morphological findings, we stated "Some rats on this same diet developed hemorrhage and necrosis of the liver. This necrosis appears to be identical with that seen by Gyorgy and Goldblatt.³ It is primarily coagulative in type and centrilobular in location and is nearly always accompanied or even replaced by hemorrhage of quite variable extent in the necrotic areas. Sometimes only small periportal islets

TABLE 1
BASAL DIETS EMPLOYED IN CIRRHOSIS AND NECROSIS EXPERIMENTS

	Basal diet No 1	Basal diet No 2	Other basal diets
Casein	4.0%*	4.0*	4.0** to 18.0**
Cystine	—	0.04%	—
Salt mixture†	4.0	4.0	4.0
Cod liver oil	2.0	2.0	2.0
Vegetable oil‡	3.0	3.0	3.0
Corn starch	87.0	86.96	—
Sucrose	—	—	to 100

* Leached

** Leached and hot alcohol extracted

† Osborne and Mendel

‡ Wesson oil

of surviving liver cells remain. When this picture and the hepatic cirrhosis described by us⁴ occur together they appear to be essentially unrelated to each other, the hemorrhage and necrosis being superimposed on almost any phase in the development of the cirrhosis. Rarely is there any evidence of the hemor

is shown in

TABLE 1. It consisted of leached casein 4 per cent, Osborne and Mendel salt mixture 4 per cent, cod liver oil 2 per cent, Wesson oil 3 per cent, and corn starch 87 per cent. A vitamin supplement, consisting of 100 micrograms of

ginal in vitamin E. The most severe amino-acid deficiencies were probably of the sulfur containing cystine and methionine, although threonine was also severely limiting for growth. At the time these experiments (summarized in TABLE 2) were carried out we were greatly interested in the effect of alcohol consumption of the exacerbation of the cirrhotic process. For this reason this particular group of animals received, from the time they were placed on the experimental diet at weaning, 20 per cent alcohol as the sole source of fluid. The effect of methionine, you will note, is compared in the table in each case with that of a somewhat lower percentage of cystine. This repre

As was pointed out

sions are clearly dif

els of added cystine

the incidence of cirrhosis increased while the incidence of necrosis (except at the 0.04 per cent level) decreased. Choline chloride (20 mgm per rat per day) protected against cirrhosis but not against necrosis. Methionine, being a precursor of both cystine and choline, protected against both types of lesions when given in sufficient amounts. The only exceptions to the general rule were three animals receiving choline and cystine. Two of these died after 201 and 245 days respectively, on experiment and showed slight liver cirrhosis. The third died after 296 days on experiment and showed hemorrhage and

Daft Liver Necrosis and Cirrhosis

625

DIFFERENTIATION OF DIETARY DEFICIENCIES IN EXPERIMENTAL CIRRHOSIS AND NECROSIS
(20% ALCOHOL AD LIB)

	No of rats	Curr %	Nec. %	Neg %
Basal diet No 1	16	19	37	62
Basal diet No 1 + Cystine 0.04%	17	53	59	24
Basal diet No 1 + Cystine 0.04% and Choline*	10	0	60	40
Basal diet No 1 + Meth 0.05%	27	19	41	32
Basal diet No 1 + Cystine 0.08%	9	100	22	0
Basal diet No 1 + Cystine 0.16%	12	92	0	8
Basal diet No 1 + Meth 0.20%	4	0	0	100
Basal diet No 1 + Cystine 0.50%	15	90	0	10
Basal diet No 1 + Cystine 0.50% and Choline*	10	13	7	80
Basal diet No 1 + Meth 0.70%	94	0	0	100

* 20 mg choline chlor de per rat per day

necrosis It is worthy of note that 0.05 per cent of added methionine was insufficient to protect against either cirrhosis or necrosis and that 0.01 per cent added cystine appeared, if anything, to increase the incidence of necrosis as in cirrhosis

rior to this work of ours in 1942, several important researches in the field of dietary liver damage had been carried out Weichselbaum,* in 1935, had reported coma and death in rats deficient in cystine and methionine and had stated that post mortem examination showed definite hemorrhages throughout the liver

No histological examinations appear to have been made but an excellent clinical description of the deficiency state was included in this report Gyorgy and Goldblatt,* in 1939, published a detailed description of the lesion, and in the same year du Vigneaud and co workers* described hemorrhage and cell damage in the livers of rats which had received diets deficient in the sulfur containing amino acids In none of these early experiments quite naturally, can the amount of choline which the rats received either from the diet or from vitamin supplements be stated with any great degree of certainty From the descriptions of the lesions, however, it appears that no hepatic cirrhosis was seen by Weichselbaum or by du Vigneaud and associates Gyorgy and Goldblatt, however stated that cirrhosis occurred with necrosis in their animals

Almost simultaneously with the publication of our differentiation between dietary cirrhosis and dietary necrosis in 1942 Gyorgy and Goldblatt reported data very similar to those which we presented with, however a different interpretation of the experimental results The following year, Hock and Fink* described liver necrosis in rats given a diet in which yeast, in rather large amounts, served as the sole source of protein These investigators reported that the inclusion of cystine in this yeast diet prevented the development of the hepatic necrosis This finding furnished new evidence that dietary necrosis is a separate and distinct entity from dietary cirrhosis

A most important finding not only to the differentiation of these two types of lesions but also to the whole understanding of the influence of dietary factors on their lack on the integrity of the liver, came in 1944 At this time it was

reported by Schwarz⁸ that the dietary liver necrosis which he had observed in many hundreds of animals over the previous several years could be prevented by the administration of vitamin E. His method of developing liver necrosis in rats was to give them a diet containing 15 per cent of alkali treated casein as the sole source of protein. There was a high incidence of liver necrosis with no liver cirrhosis. The prevention of this lesion by vitamin E together with the more recent report by Schwarz⁹ in 1951 of the existence of a third factor effective against dietary liver necrosis, strengthens still further the case for the etiological and morphological separation of the two types of liver lesions.

By 1944 then dietary cirrhosis and dietary necrosis had been fully described, a clear differentiation of the two types of lesions had been made, both on morphological grounds and on the basis of their separation by dietary means, and diets had been described on which necrosis alone developed. In addition, it was in that year that the identification of a second factor against dietary necrosis, vitamin E, was announced.

Before leaving the historical part of this presentation, however, perhaps one additional fact should be recorded. Himsworth and Glynn,¹⁰ in 1944 published the first of their series of papers in which they corroborated many of the findings of earlier investigators. In the same year, Glynn and Himsworth¹¹ described in some detail an extensive postnecrotic scarring which followed massive necrosis in some of their rats. This lesion had not been observed previously and indeed cannot easily be obtained with the diets which were in use in most laboratories at that time. It is particularly important to make this clear since Himsworth and associates appear not to have claimed the priority which is their due for this interesting observation. They appear, instead, to have credited various American pathologists with including it in the earlier descriptions of cirrhosis.

TABLES 3 to 5 show the results of some further studies in our laboratory. They were carried out in collaboration with Doctors Kenneth Endicott and Ralph Lillie who made all of the histological examinations. The basal diet and the vitamin supplement used in these experiments were the same as those

TABLE 3

DIFFERENTIATION OF DIETARY DEFICIENCIES IN EXPERIMENTAL LIVER CIRRHOSIS AND NECROSIS (WATER AD LIB)

Basal diet No. 2		+ Choline*		+ Cystine†		+ Choline* + Cystine†	
Cirr	—	—	—	Cirr	—	—	—
Cirr	—	—	Nec	Cirr	—	—	—
—	Nec.	—	Nec	Cirr	—	—	—
Cirr	—	—	—	Cirr	—	—	—
Cirr	—	—	—	—	—	—	—
—	Nec.	—	—	Cirr	—	—	—
Cirr	Nec	—	Nec	Cirr	—	—	—
Cirr	Nec	—	Nec	Cirr	—	—	—
—	—	—	—	Cirr	—	—	—
—	Nec	—	—	—	—	—	—

* 20 mg. choline chloride per rat per day

† Additonal 0.12% cystine in diet

DIFFERENTIATION OF DIETARY DEFICIENCIES IN EXPERIMENTAL LIVER CIRRHOSIS AND

Basal diet No. 2		+ Choline**	+ Cystine†	+ Choline** + Cystine†
Ctrl	—	—	Ctrl	—
Ctrl	Nec	Nec	Ctrl	—
Ctrl	—	—	Ctrl	—
—	Nec.	—	Ctrl	—
—	—	Nec	Ctrl	—
Ctrl	Nec	Nec	Ctrl	—
Ctrl	Nec	Nec	Ctrl	—
—	Nec	Nec	Ctrl	—
—	—	Nec	Ctrl	—
—	Nec.	—	Ctrl	Nec.
—	—	Nec	Ctrl	—
—	—	—	Ctrl	Nec.
—	—	—	Ctrl	—

* 20% alcohol ad lib
 † 20 mg choline chloride per rat per day
 ‡ Addit oral 0.12% cystine in diet
 § Fed after 2 and 15 days respectively

In earlier studies except that 0.05% amount of corn starch was given.

20% alcohol ad lib
20 mg choline chloride per rat per day
Additonal 0.12% pyridine in diet
Died after 3 and 15 days respectively

in earlier studies except that 0.04 per cent of cystine replaced an equivalent amount of corn starch in the diet (basal diet No. 2). It appeared from the previous small experiment (TABLE 2) that this level of cystine might lead to an increased incidence of hepatic lesions. The findings for individual animals are presented in TABLES 3 and 4 in order to show that an occasional rat had neither type of lesion while several had both types. The results in TABLE 3 clearly portray the effect of choline in suppressing cirrhosis but not necrosis, the effect of cystine, even this additional 0.12 per cent in preventing necrosis, while increasing or at least not decreasing the incidence of cirrhosis, and the effect of the combination in preventing both types of lesion. TABLE 4 gives the results obtained with a similar group of animals which received approximately 20 per cent of alcohol as the sole source of fluid. The results are very similar to those in TABLE 3, except for the higher incidence of lesions in some groups and particularly the fact that four animals receiving the higher level of cystine, three of which were receiving choline as well, died with liver necrosis. The possibility that the ingestion of alcohol may increase the incidence of this lesion probably deserves further study. It is interesting that the animals receiving choline had a slightly higher incidence of necrosis than those not receiving this supplement. The only animals receiving the basal diet plus choline which did not develop this lesion were two animals which died after 5 and 15 days, respectively, on experiment. The four animals on the basal diet which did not develop the lesion died after 42, 81, 98, and 110 days, respectively, on the experiment. TABLE 5 summarizes the results in TABLES 3 and 4 and permits a comparison of the findings with water and 20 per cent alcohol, respectively, as the sources of fluid.

All of these experiments were carried out with casein at a dietary level of 4 per cent as the sole source of protein. In our experiments, as in those of other investigators, the development of dietary liver cirrhosis and the development of dietary liver necrosis are both dependent to a large extent on the level of protein in the diet. The reason for this is clear since casein as usually

TABLE 5
SUMMARY OF TABLES 3 AND 4

	No. of rats	Cir	Nec	Neg
Basal diet No 2 (water)	10	6	5	1
Basal diet No 2 (alcohol)	10	5	6	2
Basal + Choline* (water)	10	0	4	6
Basal + Choline* (alcohol)	10	0	8	2
Basal + Cystine† (water)	10	7	0	3
Basal + Cystine† (alcohol)	10	10	1	0
Basal + Choline* and Cystine† (water)	10	0	0	10
Basal + Choline* and Cystine† (alcohol)	10	0	3	7

* 20 mg. choline chlor. de per rat per day

† Additional 0.12% cystine in diet.

TABLE 6
EFFECT OF PROTEIN LEVEL

Casein	No. of rats	Fatal kidney lesions %	Cir %*	Nec %*	Neg %*
4%	10	0	40	20	50
6%	10	0	40	50	20
8%	12	8	32	55	0
10%	12	42	100	43	0
12%	21	24	44	25	56
14%	11	27	25	0	75
16%	11	36	0	0	100
18%	11	18	0	0	100

* Of those not dying of kidney lesions

prepared, even so-called "vitamin free" casein, has been shown by Schwarz to contain Factor 3, which prevents liver necrosis, and of course contains also cystine and methionine. Both of these amino acids, as has been indicated, help to prevent the necrosis while methionine is very active in the prevention of cirrhosis. TABLE 6 gives some of our data on this point. In this group of experiments, the diets which were used contained leached and hot alcohol extracted casein, 4 to 18 per cent, salt mixture 4 per cent, cod liver oil 2 per cent, Wesson oil 3 per cent, and sucrose to make up the balance. Vitamins were given as in the earlier experiments. Water was the source of fluid. A number of these animals died from the acute kidney lesions first described by

cent, no cirrhosis on levels of casein above 14 per cent, and no necrosis on levels of casein above 12 per cent. In passing it might be noted that the suggestion has occurred frequently in the literature that rapid growth is essential for the development of kidney lesions. Rapidity of growth is certainly a factor, but we have had no trouble in obtaining fatalities from this lesion routinely on

Daft: Liver Necrosis and Cirrhosis

629

TABLE 7
CIRRHOSIS "SCORE" AS AFFECTED BY RIBOFLAVIN INTAKE

R. bioflavin level (micrograms per rat per day)	Experiment number				
	1	2	3	4	5
5	2 0	2 3	2 3	2 0	1 6
50	1 2	1 4	1 6	1 3	1 1
Exp 1-3	No choline added to diet				
Exp 4-5	25 mg choline chloride/100 g diet				

diets too low in protein to permit growth, by including cystine, high levels of fat, and cholesterol in the diet.

I should like to pass now to some studies on the effect of a low riboflavin intake on the development of liver cirrhosis in rats which were carried out in collaboration with Doctor George Fite. These studies were prompted in part by the observations concerning "yellow liver" in dogs by Sebreli and Onstott¹² in 1938. In the light of information accumulated in large part since their experiments were carried out, it appeared possible that the condition they described might have developed as a result of combined riboflavin and choline deficiencies. There were also the numerous clinical studies using diets similar to the high protein, high vitamin diet of Patek and Post¹³ which seem to indicate that nutritional deficiencies other than that of choline are of importance in clinical liver cirrhosis. In addition, there is the well known protection of the liver from certain toxic materials such as butter yellow¹⁴ by an increased intake of riboflavin. The diets used in these experiments contained so-called "vitamin free" casein (G B I) 10 per cent, a modified Osborne and Mendel salt mixture* 4 per cent, cod liver oil 2 per cent, Wesson oil 3 per cent, and sucrose 81 per cent. The diet of groups 4 and 5 in TABLE 7 contained also 25 mgm per cent of choline chloride. Two vitamin supplementations were used, the one which I have previously described and another which differed only in that the riboflavin level was reduced from 50 mg to 5 mg per rat per day. At weaning, Osborne and Mendel rats were divided equally on a litter and sex basis and maintained under the experimental conditions described for one year unless death or sacrifice of the animals, histological examinations of 176 rats. Following death or sacrifice of the animals, the number of rats were made by Doctor Fite. Each liver was given a score, 0 if no cirrhosis could be observed, or 1, 2, or 3, if cirrhosis was observed, the number assigned depending on the severity. These figures were then averaged for the group. The higher figures in the table, therefore, represent the greater average incidence and severity of cirrhosis. It appears that the animals which received the 5 mgm level of riboflavin in all experiments had a greater degree of cirrhosis than their litter mates which received 50 mgm of this vitamin daily. The probability of such an outcome due to chance alone in 5 consecutive experiments is 1 in 32. The result is significant at the 0.3 level. On the

* Prepared according to the diet recipe of Osborne and Mendel except that the sodium fluoride is reduced to 1 per cent of their level and 0.315 gm of CuSO₄ · 5H₂O (equivalent to 0.7 g anhydrous CuSO₄) is added.

TABLE 8

EFFECT OF COMPOSITION OF FAT COMPONENTS OF DIET ON DEVELOPMENT OF KIDNEY LESIONS

	No. of rats	Acute lesions	Healing or scars	Neg.
		%	%	%
Stearic acid	67	52	24	24
Palmitic acid	49	39	20	41
Oleic acid	48	0	17	51
Soybean oil	8	0	0	100
Soybean flakes	8	37	25	37

basis of these results we should like to propose that the level of riboflavin in a diet, not completely adequate in other factors, may influence the development of liver cirrhosis

Finally, I should like to present the results of some experiments on the effect of the composition of the fat component of the diet on the development of fatty livers and on the development of kidney hemorrhage and necrosis. These studies were carried out in collaboration with Doctor Kenneth Endicott who made all of the histological examinations. The general plan of the studies was for weanling Wistar or Sprague Dawley rats to be divided according to sex and litter and given one of three experimental diets. These diets contained casein, which had been leached and extracted with both cold and hot alcohol 4 per cent, modified Osborne and Mendel salt mixture 4 per cent, cystine 0.5 per cent, sucrose 81.5 per cent, and palmitic, stearic, or oleic acid 10 per cent. In a small experiment covered by the last two lines in TABLE 8, soybean oil, or soybean flakes which were prepared by the hydrogenation of this particular lot of soybean oil, was substituted at the same level for the 10 per cent of fatty acid. Both sexes of rats were used, the ratio in each group being roughly three males to one female. In the case of 36 animals, 12 from each of the first three groups, the experiment was terminated after 10 to 14 days and the livers were analyzed for total fat. In the case of 35 additional animals in these three groups, the experiment was terminated by sacrifice of the animals at 16 to 21 days. The remaining 93 rats divided between the three groups were kept on these three diets until their deaths which occurred after experimental periods ranging from 11 days to 216 days. As may be seen from the table, the incidence of acute kidney lesions ranged from 0 per cent for those receiving oleic acid to 39 per cent for those receiving palmitic acid and 52 per cent for those receiving stearic acid. Were we to exclude the animals which were sacrificed, the results would be even more striking. None of 22 animals on oleic acid, 52 per cent of 29 animals on palmitic acid, and 69 per cent of 42 animals on stearic acid showed acute lesions when the experiments were permitted to proceed to the death of the animal. It will be noted further that the results of a small experiment, carried out with unhydrogenated soybean oil and with the same lot of oil hydrogenated to a form known as soybean flakes, support the idea that the degree of saturation of the fat or fatty acid which makes up the fat portion of the diet influences very greatly the incidence of this fatal acute

Daft Liver Necrosis and Cirrhosis

631

TABLE 9
LIVER FAT AS AFFECTED BY NATURE OF FATTY ACID INGESTED

Fatty Acid	No of rats	Fat	
		%	Range
Palmitic	9	25.4	(12.0-38.0)
Stearic	9	18.3	(13.4-27.9)
Oleic	9	16.2	(11.2-25.0)

TABLE 9 gives the fat analyses of the livers of nine groups of litter mates. There appears to be little difference in the fat content of the livers of the rats receiving oleic or stearic acid. The level of liver fat of the rats which received palmitic acid appears to have been somewhat higher.

An insufficient number of the 93 animals survived the acute kidney lesions in this series of experiments to permit us to draw any definite conclusions as to the incidence and severity of cirrhosis. I shall merely mention that of the animals thus surviving one out of 13 or 8 per cent on stearic acid, three of the or 21 per cent on palmitic acid, and six of 22 or 27 per cent on oleic acid were found to have some degree of cirrhosis. The oleic acid animals had not only the highest incidence of cirrhosis but also showed the more severe lesions. These fatty acids, as might have been expected from our earlier work,¹⁶ appeared to be very poor precursors of ceroid since even in those livers which were cirrhotic there was either none of this pigment or only a trace. One rat receiving palmitic acid and one receiving oleic acid showed massive liver necrosis.

In conclusion, we should like to suggest on the basis of these results that the nature of the fatty acids in the fat component of the diet deserves consideration in studies of fatty livers, acute choline deficiency, and hepatic cirrhosis. It will be of interest to determine whether or not the nature of the fat component of the diet is of importance in the development of liver necrosis as well.

References

1. DAFT F S, W H SEBRELL & R D LILLIE 1942 Federation Proc 1(2) 183
2. DAFT F S, W H SEBRELL & R D LILLIE 1942 Proc Soc Exptl Biol Med 60 1
3. GORDON P & H GOLDBLATT 1939 J Exptl Med 70 183
4. LILLIE R D, L L ASHURN & W H SEBRELL, F S DAFT & J V LOWRY 1941 Pub Health Repts 57 80
5. WEICHELBAUM T E 1935 Quart J Exptl Physiol 25 363
6. DO VIGORATO V, H M DYER & M W KIES 1939 J Biol Chem 130 325
7. HICKS A & H FINK 1943 Z physiol Chem 281 109
8. SCHWARTZ A 1931 Z physiol Chem 278 136
9. SCHWARTZ A 1931 Proc Soc Exptl Biol Med 78 832
10. HILKIN W H & L E GUYON 1944 Clin Sci 5 93 133
11. HILKIN W H & L E GUYON 1944 J Path Bact 56 297
12. GUYON L E & H P HILKIN 1943 J Biol Chem 131 567
13. SEBRELL W H & J WADE 1939 J Path Bact 56 297
14. SEBRELL W H & J WADE 1941 J Clin Invest 20 481
15. KESLER C J & K SUGITA A F 1941 Proc Soc Exptl Biol Med 67 330
16. DAFT F S, W H SEBRELL & C P REARDS 1941 Science 93 308-310
17. ENNOCOTT A M, F S DAFT & W H SEBRELL 1941 Proc Soc Exptl Biol Med 67 330

Discussion of the Paper

DOCTOR PAUL GYORGY (*School of Medicine, University of Pennsylvania Philadelphia Pa*) The distinction of acute hepatic necrosis from chronic fibrosis, as first proposed by Doctor Daft and Doctor Sebrell, has proved to be a very useful and fruitful concept. In experimental hepatic injury in rats, the etiologic dietary factors for necrosis and cirrhosis appear to be definitely different. In our own laboratory, we have observed independently of Doctor Daft and Doctor Sebrell the beneficial effect of cystine and methionine in experimental necrosis of the liver and the injurious effect of cystine regarding cirrhosis. Nevertheless we have abstained from a clear cut separation of necrosis from cirrhosis for two reasons (1) In toxic hepatic injury, the hepatotoxin in large doses may produce necrosis and, in small repeated doses typical Laennec's cirrhosis, and (2) Cystine in amounts not much in excess of the therapeutic dose range will not only prevent but produce hepatic necrosis.

Later, it has been found, first by Schwarz, then independently in our laboratory, that hepatic necrosis is preventable not only by sulfur-containing amino-acids but also by vitamin E. This raised the question whether necrosis is a simple or conditioned deficiency.

THE SEQUENCE OF PATHOLOGIC EVENTS IN THE DEVELOPMENT OF EXPERIMENTAL FATTY LIVER AND CIRRHOSIS*

By W. Stanley Hartroft

Bunting and Best Department of Medical Research, University of Toronto, Canada

Introduction

Interest in the fatty livers which may be produced in experimental animals by feeding diets low in choline and its precursors (betaine, methionine and methionine-containing protein) has extended in our laboratories at Toronto over a period which can be said to have originated with the discovery of insulin in 1922 and has continued up to the present time. The sequence of pathologic events which begins almost as soon as an animal is placed on the low-choline regimen has now been studied in a large number of animals. Most of the early studies which resulted in the discovery by Best and his associates² of the hypotrophic action of choline were carried out on dogs, but since the end of World War II most of our experiments concerning the pathological changes in choline deficient animals have been carried out on the rat. Other species of animals are similarly affected as will be reviewed by Professor Best and his co-workers in the next paper. Much of the available evidence indicates that man should probably be included in the list of animals susceptible to some of the pathologic effects of low choline diets.

Some of the early morphological changes which affect the liver in dietary choline deficiency are those described by Maclean and Best in 1934.¹¹ In this paper were reported the appearances of livers of dogs fed diets low in choline and of those of control animals which had received the same basal diet supplemented with adequate amounts of the hypotrophic agents. Photomicrographs were published in black and white as well as colored drawings of some of the full sections. Detailed protocols of some of the animals were published in (TEXTS 1 and 2). The intracellular storage of fat within parenchymal liver cells is well illustrated and described. In addition these photomicrographs show definite evidence of the extracellular phase of fat storage within what we call fatty cysts. Furthermore in two of the illustrations there is evidence of early fibrosis in these livers and in the protocols it is expressly stated that in some areas of the liver the number of fibroblasts and leukocytes is increased. These reports clearly foreshadowed the recognition four years later of cirrhosis in animals fed a choline deficient diet by Chaikoff and Connor¹² in dogs and by Coryell¹³ in rats.

We have begun with this description because many phases in the sequence of events which we are about to describe in the livers of choline-deficient rats were already evident in these earlier studies. In this similar phenomenon

can be studied in a greater number of animals because the lesions make their appearance within shorter periods than is the case in dogs. All the findings to be set forth briefly below have been previously published^{7, 8, 9, 11, 12} or are in press¹⁰. This paper will consist, therefore, of a summation of our conclusions based on these studies. Data concerning dietary methods, histological techniques and photomicrographic evidence are given in full in the publications mentioned.

1 Intracellular phase of the accumulation of abnormal fat in livers of choline deficient rats "Intracellular lipohepatosis"

Within a few hours after allowing rats access to diets low in choline, stainable fat appears within many of the liver cells.¹³ The fat is clearly intracellular in position and at this stage, is seen in greatest amount within parenchymal

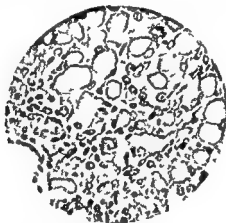


FIGURE 1. Comparison with FIGURE 2. Early fibrosis in dogs results from atrophy and fibrotic replacement of fatty cysts. FIGURE 2B in M. A. Lean and Best.¹⁴

cells in centrilobular and nonportal regions. Stainable fat droplets may not accumulate in portal liver tissue until a later stage. Even such an early manifestation of the hepatic lesions of choline deficiency is in accord with the pathonomy that the effects of this dietary abnormality are always first observed and are present in greatest degree in the nonportal regions of the liver. This principle as we shall see, holds true at all stages, including those of advanced cirrhosis.

Many terms have been used in the past to describe the abnormal accumulation of fat in the livers of choline deficient animals. Such have included fatty change, fatty infiltration and fatty degeneration. In our opinion none of these phrases is free from ambiguity when used to describe the lesions in hepatic hypocholeminopathy. Fatty change might imply merely a change in the state of the liver lipids, but we know¹ that the fatty droplets represent an accumulation of excess fat brought to the liver from the ingested food. The

fat is initially held within liver cells and not in adipose tissue cells between them, as the term fatty infiltration would imply.

Since we suggest the term

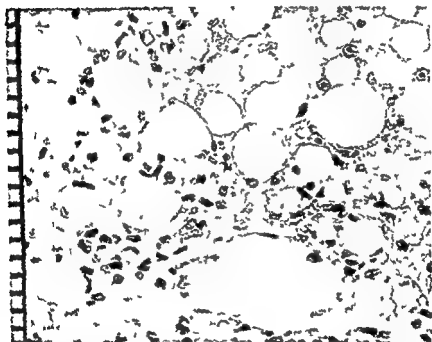


FIG. 10. (Continued from page 634) Region of hepatic infarction and necrosis. Paraffin section, hematoxylin and eosin stain.

lip hepatitis for this condition. The phrase described in this section is that of intercellular lip hepatitis. This is followed by extracellular lip hepatitis of which the original feature is the formation of fatty cysts.

Extracellular lip hepatitis formation

It is to be noted that in this condition the liver is free in all respects from water, which thus escapes from within the liver and is therefore contained in relatively large pools of lipid within simple epithelial cysts. The cysts are

formed in the following manner. Progressive enlargement of fat globules within neighboring liver cells compresses the adjacent intervening segments of their limiting cell membranes (which have been caught between the globules) into morphologically single, tenuous septa. By its formation such a septum conjoins the parent cells but still separates the adjacent fat globules and prevents them from coalescing. If the globules continue to enlarge they eventually stretch the interposed septum that it is torn. As a result, the fat globules in the torn cells coalesce. The resultant pool of fat is enclosed, however, by the crescentic remnants of the cytoplasm of the parent group of cells which are still linked together. The nuclei of the cells appear essentially unaltered due to the fact that the limiting membrane is not torn.

The resulting structure is a cystic space filled with fat, surrounded by liver tissue but is contained within a simple, epithelial cyst. Fatty cysts once

pathological routes to be described. When fat is no longer either intracellular or intracystic, it appears to constitute a grave menace to the health of the animal.

3 *Extracellular lipohepatosis: rupture of fatty cysts and formation of trabeculae*

It has been observed repeatedly that the distribution of fatty cysts in the livers of choline deficient rats presages that of the fibrous trabeculae which are formed later. This juxtaposition is not merely fortuitous for the evidence clearly indicates that it is the dissolution of ruptured cysts that produces the trabeculae. Fatty cysts form initially, as already mentioned, around the centrilobular radicles of the hepatic vein. From here they extend in annular chains which link adjacent central veins. They apparently follow this course by virtue of the fact that if they extend into any other portion of the lobular parenchyma they will be advancing more or less directly towards nearby portal areas. The progressive sites in which fibrosis develops are identical with those in which cysts previously have formed. Trabeculae result from condensation of the parenchymal and stromal remnants of ruptured fatty cysts. This condensation of cyst remnants occurs whenever the cysts lose their fatty contents. Rupture of cysts over 100 micra in diameter (as measured in paraffin sections) is apparently nearly always accompanied by escape of fat. Large cysts are covered with a fine network formed of bile capillaries and sinusoids which originally ran between the liver cells that have taken part in the formation of the cyst wall. The larger the cyst the more extensive this network, and accordingly the more likely that some component of the latter will be torn with rupture of the cyst wall. As a result free communications may be established between the lumen of the cyst and those of sinusoids or bile ductules. Fat may be drained from the cyst by either of these two routes, and the remnants of the emptied structure condense into a fibrotic residuum. In this manner the annular chains of cysts connecting adjacent centrilobular regions progress

Hartroft Fatty Liver and Cirrhosis
into the fibrous trabeculae familiar to anyone who has observed early stages
of experimental dietary cirrhosis in animals

637

4 The development of a "portal" distribution of the fibrous tissue

In their initial reports Gyorgy and Goldblatt¹² interpreted the distribution of fibrosis in choline deficient rats as portal. They were studying late stages of the condition. Although it is now clear from the work of Lillie *et al.*,¹⁴ Gibbon *et al.*,² and from the foregoing description that in the early stages, the trabeculae are nonportal, later fibrosis clearly develops around large, easily identified portal trunks. In this sense the cirrhosis is unquestionably portal in distribution. But the fibrosis in these sites replaces parenchyma which although adjacent to conducting portal vessels (Elias) is not perportal in the sense that it is supplied by blood which has not previously come into intimate contact with liver cells. Parenchyma that lies beside these large conducting veins is supplied by sinusoids which do not frequently or necessarily communicate directly with the near by veins. In most instances it has been shown¹⁰ that these sinusoids receive their blood via others that in turn communicate with terminal venules, which actually perform the function of distributing the blood to the liver parenchyma. The large conducting veins are those which bring the blood to the terminal venules, and the latter empty the blood into sinusoids which rarely communicate directly with vessels of the order of size of veins. This is generally true of tissues and organs throughout the entire body for example in the kidney and pancreas capillaries and sinusoids are always in the center of a structural unit of liver tissue described by Rappoport and his co-workers as the hepatic acinus.¹⁵ The periphery of an acinus may sometimes lie against a conducting vein and hence parenchyma in such a site although perportal in a geographic sense receives its blood via other sinusoids from a terminal venule. Such sites are in terms of the entry of fresh blood into a hepatic unit no more functionally perportal than are sites which are obviously closer to radicles of the hepatic vein. Fatty sites and subsequently their trabeculate remnants may therefore appear in these regions adjacent to conducting veins. Fibrosis in such positions at first appeared to constitute a paradoxical exception to the postulated distribution of fibrosis in choline deficiency. If the hepatic architecture is interpreted according to Rappoport's concept of the hepatic acinus, and in terms of the functional entry of fresh blood into sinusoids around terminal distributing venules the paradox is resolved. It then becomes clear why initially nonportal lesions between geographically isolated perportal and surrounding large conducting portal trunks. The latter structures are prominent even under the lower powers of the microscope and unless the sites of the smaller relatively inconspicuous terminal venules are examined with the aid of higher magnifications it may not be realized that the silicified parenchyma which is both geographically and functionally perportal is free from fibrosis. The term portal cirrhosis is applicable at this stage from a purely descriptive standpoint but it should be

realized that the sites of fibrosis are actually nonportal with reference to blood supply. In microsections of cirrhotic livers obtained at autopsy from alcoholic patients we have observed that the parenchyma around terminal portal venules is usually quite free of fibrotic lesions,¹⁰ although the large conducting veins in these cases frequently were surrounded by pathological amounts of fibrous tissue. This suggests that the distribution of the trabeculae in alcoholic cirrhosis in man is also periportal only in a geographic sense and that in this respect as well as perhaps in others dietary cirrhosis in choline deficient rats is the morphologic counterpart of that found in alcoholic man.

5 Nodular hyperplasia

After four or five months of choline deficiency, focal areas of hyperplasia throughout the liver become prominent features in microsections. In these areas which surround the terminal portal venules, mitotic figures may be found with ease. Frequently, cells with two, three, or even four nuclei may be encountered. As a result of this proliferation nodules of compensatory parenchymal tissue are formed and they project, on the surface of the organ, thus producing the characteristic hobnail appearance which is such a striking feature of these livers when examined grossly. Growth of the nodules results in com-



FIGURE 3. Advanced hyperplasia of bile ducts and regenerating liver cells in choline deficient rat's liver in stage of late nodular cirrhosis. This field may represent precancerous or early neoplastic change. Paraffin section, hematoxylin and eosin stain. Divisions of scale are 10 microns apart.

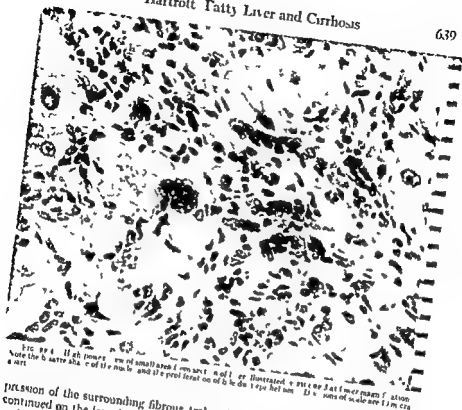


FIG. 39. High power view of small areas from section of liver illustrated in figure 38. Note the better preservation of the nodules and the proliferation of the ductal system. Dimensions of scale are 1 mm.

pression of the surrounding fibrous trabeculae. Even though the animals are continued on the low choline regimen the newly formed tissue rarely becomes as fatty as that previously present. In the regions of compensatory proliferation the terminal vessels may lengthen and divide. Studies of the arterial supply of the large nodules have not yet been made.

6 Neoplasia

At the edges of the nodules as well as within their substances in some instances isolated liver cells may assume bizarre forms with rather wide variations exhibited in their size, shape and staining reactions (Figures 40 and 41). In our experiments this appearance rarely suggests anything more than regenerative hyperplasia. There is however little doubt that in susceptible strains of rats cirrhosis induced by low-choline diets may progress to true neoplasia. The evidence for hepatic tumor formation in choline deficient rats is presented in a paper by Doctors Copeland and Salmon who first reported its occurrence.

Conclusions

The escape of fat from cells into the vascular system results in intermittent or chronic episodes of fat embolism. These emboli are carried to the heart.

lungs, and kidneys where microscopic evidence of their damaging effect has been noted^{1, 2}. Other organs may well be involved in this phenomenon, and it is possible that the arteriosclerotic changes in the aorta, carotid arteries, and coronary vessels which we have reported in choline deficient rats¹² may be another result of fat circulating in the blood stream in this obviously pathologic state.

We have followed the regressive changes which regularly develop in fatty hepatic cysts of previously choline deficient rats when lipotropic factors are restored to the animals' diets¹³. Intracellular fat is mobilized within a matter of hours, thus rendering the cysts particularly prominent. Weeks, and, in some instances, months, of choline administration may be required before the fat within cysts is absorbed by the cells forming their walls. Once lipid regains this intracellular position, dietary choline rapidly effects its disappearance. The fact that cysts may remain for relatively long periods in previously fatty livers even after therapeutic measures have removed all intracellular, stainable fat offers a diagnostic aid in the identification of fibrosis of those types which may have been initiated by lipohepatosis. We have examined sections from one hundred consecutive, unselected cases of cirrhosis coming to autopsy in the Department of Pathology at the University of Toronto, in an effort to evaluate the possible diagnostic significance of residual cysts within fibrous trabeculae. Almost without exception, these intratrabecular cysts were present in cases of cirrhosis in which the history clearly indicated that they had been associated at some time with lipohepatosis.¹⁴

Fatty cysts are the cytometa-plastic links between lipohepatosis and cirrhosis. In miniature findings in livers of increasingly fatty, fibrous formation and rupture of fatty cysts is by condensation of their remnants, the direct cause of fibrosis. As the livers become increasingly fibrotic their total fat content, as assayed biochemically, decreases. This phenomenon is explained by the concept that the formation of the fibrous trabeculae is dependent on the escape of fat from the cysts and thus eventually from the liver via either the biliary or vascular systems. These events would lead one to expect the total fat content of the liver to vary inversely with the amount of fibrous tissue as is the case. This relationship is probably emphasized by the fact that new tissue in regeneration nodules rarely undergoes appreciable fatty change. The concept of the formation and natural history of fatty cysts permits an appreciation of the pathogenic relation between excessive fat and fibrosis in livers. Formation of fatty cysts and condensation of their ruptured remnants into fibrous trabeculae implies *ipso facto* that the fibrosis is the direct result of overloading focal regions of the liver parenchyma with abnormal fat. It is apparent from this that the mere presence of even appreciable amounts of fat in the liver will not necessarily lead to fibrosis. Fibrosis will result only if the accumulation of fat is so great that rupture of cells produces cysts which in turn, disintegrate. This explanation may serve to help resolve some of the apparently conflicting conclusions which have been previously drawn concerning the cirrhotogenic nature of lipohepatosis.

liver studied by serial biopsies which at the end of two or three years still failed to show significant portal fibrosis let alone cirrhosis

(3) In our experience, fatty metamorphosis of the liver in any one patient may come and go without appreciably affecting the degree of portal fibrosis or cirrhosis

factors which causes the necrosis also happens to cause fatty metamorphosis. Fatty metamorphosis without the basic factor of liver necrosis does not lead to fibrosis. On the other hand, we have seen extensive necrosis of liver without appreciable fatty metamorphosis yet this will be followed by cirrhosis.

This type of cirrhosis comes about as the result of two main processes in crease of collagen and nodular regeneration of liver cells. (a) The diffuse necrosis of liver leads to a fairly diffuse but somewhat irregularly distributed increase in collagen not only in portal areas but particularly in the parenchyma which it traverses in thin bands. (b) In the absence of the original reticular framework to guide the regenerating liver cells the newly formed parenchyma grows in the form of roughly spherical nodules without proper relationship to pre existing key structures such as portal areas and central veins. Indeed the intralobular bands of collagen may be partly responsible for this abnormal architecture by forming a new but haphazard framework along which the regenerating tissue grows.

(5) The acute necrotizing phase is characterized by a fairly diffuse degeneration and necrosis of liver cells many of which are transformed into Mallory bodies. Accompanying this is a moderate amount of inflammatory reaction scattered throughout the lobules and involving portal areas as well. Fatty metamorphosis may or may not be present. At this point three things may

Doctors Hartroft and Dubin. Studies with Doctor Hans Elias based on three dimensional reconstruction and geometrical analysis have convinced me that there are several pathways along which fatty liver may proceed to cirrhosis formation. One pathway is that which Doctor Hartroft has explored by his thorough and ingenious investigations. I feel however that this occurs

from collapse of the fatty cysts or from the formation of collagen fibers in the vicinity of the cysts. The second pathway is the result of necrotizing and inflammatory changes complicating the fatty liver, somewhat similar to the

Hartroft Fatty Liver and Cirrhosis

643

processes which Doctor Dubin just described so well. The inflammation or the necrosis with associated inflammation leads to an irregular fibrosis with the formation of collagen fibers in the parenchyma. Moreover, radiating or stellate fibrosis of the portal tracts develops. The latter may possibly be due to irritation by breakdown products drained from the parenchyma to the portal tracts. In our opinion, however, the most important pathway results from stress within the parenchyma exerted from the vicinity of regenerative, ordinarily fat free, liver cell plates, usually several cells thick, and the fat containing one cell thick plates. This stress produces planes of breakage in which fibers develop to form a straight septum, subdividing the lobule. In human fatty livers, where periods of nutritional disturbance alternate with at least partial recovery, these stress planes probably develop more readily. In conclusion, I believe that the degree of participation of the different pathways varies in the individual cases. This accounts for the polymorphic picture seen in the development of cirrhosis from the fatty liver in man.

DOCTOR CHARLES S. DAVISOV (*Thorndike Memorial Laboratory, Boston City Hospital, Boston, Mass.*) In studying alcoholics with acute liver disease at the Boston City Hospital, we have observed ubiquitous fatty changes in the livers but we are not convinced that the fatty changes always constitute the most important pathological abnormality. A typical hepatic lesion characterized by parenchymal disorganization, intracellular hyalin and necrosis frequently occurs and is distinct from the fatty lesion. This lesion correlates better than does fat with the acute severe liver impairment which often leads to fatal coma. The lesion seems to have a predilection for females. A summary of this work done in collaboration with Doctor Gerald G. Phillips, Dante Campagna Pinto, and Frederick Parker Jr., was published in the *Proceedings of the American Federation for Clinical Research* meeting for May 1953.

DOCTOR HARTROFT In reply to Doctor Dubin's comment I should like to emphasize the following points. We have examined nearly one hundred cases of cirrhosis in human patients and in a very high percentage of cases associated with alcoholism, diabetes obesity and anemia fatty cysts in various stages were found within fibrous trabeculae. Accordingly we have named cysts in this position *intra-trabecular fatty cysts* and from their position have concluded that as in the rat these cysts may be cirrhotic in nature. These results have already been published (*Hartroft 1953 Arch Path 55:63*).

Doctor Dubin is of the opinion that fatty metamorphosis of itself does not lead to hepatic necrosis or cirrhosis and cites instances of fatty livers which he has followed by serial biopsies and which at the end of two or three years, failed to show fibrosis or cirrhosis. We have never maintained that an abnormal accumulation of fat leads to necrosis, nor have we ever maintained that the mere presence of stainable fat in the liver leads to fibrosis. All our studies both experimental and clinical of the pathogenesis of dietary cirrhosis lead us to believe that not only must fat be present in the liver to produce fibrosis but also it must be accumulating so rapidly and to such a degree that it ruptures cells thus leading to parenchymal damage. The complete history of fatty

cysts their formation, rupture, atrophy, and condensation of stromal remnants into fibrous bands, describes a series of steps by which excessive accumulation of hepatic fat leads to traumatic rupture of distended cells and to fibrosis. We have seen many instances in rats where the conditions were such that the lipotropic deficiency, although severe enough to cause accumulation of fat in liver cells, was not sufficiently extreme to produce fatty cysts and their sequelae, including fibrosis and cirrhosis. It is equally true that, once cirrhosis is established similar degrees of "nontraumatic" accumulation of fat could come and go, as Doctor Dubin states, without appreciably affecting the degree of cirrhosis.

In the experimental animal subjected to dietary choline deficiency (uncomplicated by a concomitant deficiency of tocopherol, cystine, and methionine), there is no doubt whatsoever that a cirrhosis results. In these animals, the consensus of opinion of those experimental pathologists who have studied the livers in detail is that necrosis of parenchymal cells is hardly ever seen. We are using the term necrosis here in the sense meaning acute or sudden death of tissue. It is apparent, however, that the slow death of cells or atrophy, associated with rupture of fatty cysts is, in a sense, necrosis, if the term is used with this broad meaning. We prefer, however, for the sake of clarity, to reserve the term necrosis for the sudden acute type of cell death charac-

there

than

usual

Doctor Popper has excellently summarized the points regarding the patho-

In all attempts to correlate the amount of fatty change with the amount of fibrosis and/or cirrhosis in sections whether of the experimental animal or alcoholic man, it is well to emphasize that there is abundant evidence that the greater the fibrosis the less the amount of fat. This inverse correlation is again reflected by the "life story" of fatty cysts seen in livers of choline deficient rats. The more cysts which rupture, thus allowing their contained fat to escape from the liver by one or more of the pathological routes we have described (Hartroft W S & J H Ridout 1951 Am J Path 27: 951-991) the greater the fibrosis resulting from their condensed parenchymal and

and cirrhosis. If in addition the animal becomes sick and fails to eat for a period before death or if it is treated with adequate diet and lipotropic factors much of the fat in the cirrhotic liver will be mobilized (Hartroft & Sellers 1952 Am J Path 28 387-399). Residual intratrabeular cysts may not be numerous and almost certainly will be small, but in the experimental

Hartroft Fatty Liver and Cirrhosis

645

animal where every stage of the fat accumulation and subsequent cirrhosis has been followed, it is clear that these few persistent cysts represent the stigmata of a liver that was previously fatty to the "bursting point." Similar findings in man may have the same significance and may, therefore, provide important diagnostic evidence suggesting the etiology and pathogenesis of the cirrhosis manifest at autopsy. We believe that, if these points are kept in mind, much of the apparent confusion concerning the pathogenic relationship of fatty to cirrhotic livers might be resolved, not only in experimental animals, but also in man, including perhaps some of Doctor Davidson's cases.

THE LIPOTROPIC FACTORS

By C H Best, C C Lucas, and Jessie H Ridout

Banting and Best Department of Medical Research, University of Toronto, Canada

The nutritional role of choline was discovered^{1, 2} in 1932, so that this year it comes of age as a dietary factor. Choline was the first of the accessory food factors which was shown to protect the liver, *i.e.*, in its absence from the diet, pathological changes appear promptly in the hepatic cells. The work on choline, as a survey of the literature clearly shows, stimulated great interest in liver fat in the effect of large accumulations of fat on hepatic function, and in the part played by other dietary factors in the protection of the liver.

The very general and broad title assigned to us here has made us somewhat uncertain about what is desired. The topics to be dealt with by our learned colleagues on this comprehensive program leave us but little in the way of definite responsibility, and we are therefore quite free to discuss certain general aspects of our subject.

We are all familiar with the fact that choline, betaine, methionine and under some conditions, vitamin B₁₂ and inositol, protect rats in varying degrees against the development of fatty livers. In the absence of choline from the diet, many species of animals have been found to develop this abnormality. The rat,^{1, 2} dog,^{3, 4, 5, 6} mouse,^{7, 8} rabbit,⁹ hamster,⁹ calf,¹⁰ pig,¹¹ and duckling¹² exhibit fatty livers when the diet lacks sufficient of the lipotropic agents choline or its precursors. Some of the classical lesions are illustrated in Professor Hartroft's presentation (page 633). In some species, such as the guinea pig, it is very difficult to produce an abnormal deposition of fat in the liver by feeding diets low in choline.¹³ We must always keep in mind that interference with the function of hepatic cells by means other than deficiency of the lipotropes may lead to fatty livers. Fatty livers of guinea pigs on scorbutic diets¹⁴ are probably not attributable to a lack of dietary choline.

similar renal lesions have been reported in young pigs¹⁶ and calves¹⁰. The failure to produce this kidney lesion in young mice and puppies, both of which develop fatty livers readily enough, reveals a problem which may possibly require extended study. A comprehensive investigation and comparison of the relevant enzyme systems will undoubtedly help. An interesting sequel to the renal lesion in rats is the appearance in later life of hypertension.¹⁷ This has not yet been reported in other species. You are also familiar with the failure of baby chicks,¹⁸ turkey poults¹⁹ and ducklings^{20, 21} to grow and their tendency to develop slipped tendon disease on choline deficient diets. In our early studies on the effects of choline in preventing and curing the pathological changes in the diabetic dog, we mentioned "areas of beginning fibrosis" which did not appear to be as favorably affected by choline as the fatty changes."

but we did not observe cirrhosis until after the work of Chaikoff and Connor²³ on dogs and György and Goldblatt²⁴ on rats. The role of choline deficiency in the production of fatty livers and cirrhosis in man is less clear cut. The crucial experiment to determine the lipotropic activity of choline in man has not yet been performed. The nearest approach is that of Phillips, Gabuzda, and Davidson.²⁵

By these brief references to the effects of choline and its precursors in various species, we have been leading up to reconsideration of the word "lipotropic." What should be included under this term? The word was introduced to describe substances which decrease the rate of deposition and accelerate the rate of removal of excess liver fat and was so defined by Best, Huntsman, and Ridout.²⁶ While the word dietary was not specifically mentioned, it was obvious from the context that the active principle under discussion was a component of the diet. The word lipotropic has been carelessly used, but some of the misuse is perhaps due to real difficulties in deciding on the proper scope of the term. There is, of course, no justification whatever for many of the misapplications, e.g., a change in lipid content of a fat laden pterygium on the cornea or in the amount of depot fat in obese human subjects or in animals. The term lipotropic is not applicable to changes in lipid concentration in the blood or generally in the body. The word does not apply to increases in liver fat, which at least one writer has suggested. The original definition stated that the term refers to decreases in fat deposition and is applied to changes in the liver. At one time, however, we²⁷ suggested an extension of this to include the other established effects of choline and its precursors, viz. prevention of renal lesions and of cirrhosis. Whether this proposal was wise has since been debated many times in our laboratory. Some of these changes may include effects involving fat deposition, e.g., in the kidney²⁸ or perhaps in the walls of blood vessels. If it is firmly established that the primary, or a highly significant part of the process initiated by choline in organs other than the liver is essentially similar to that in the hepatic cells, i.e. involving the prevention of excess fatness. The term lipotropic may then be extended to include these other lesions.

Let us compare the antiperotic action of choline and its effect in preventing the renal hemorrhagic syndrome. We can find no justification for calling the antiperotic effect lipotropic, but if prevention of deposition of excess fat in the proximal renal tubules is the essential process, thus protection of the kidney may be considered a lipotropic phenomenon.

On the other hand, although the growth effect of choline, which can be clearly demonstrated under certain conditions, is mediated through biochemical processes intimately interrelated with lipotropic action, this action should be designated by a term other than lipotropic.

We should stress here that a substance may exert a lipotropic effect without being a lipotropic agent. It might increase the rate of formation of, or potentiate in some way the action of, a lipotropic agent. The role of antibiotics, such as aureomycin in lipotropic phenomena is not too clear. Doctor György will discuss these in detail. We feel that they have

THE LIPOTROPIC FACTORS

By C H Best, C C Lucas, and Jessie H Ridout

Banting and Best Department of Medical Research, University of Toronto, Canada

The nutritional role of choline was discovered^{1, 2} in 1932, so that this year it comes of age as a dietary factor. Choline was the first of the accessory food factors which was shown to protect the liver, *i.e.*, in its absence from the diet, pathological changes appear promptly in the hepatic cells. The work on choline, as a survey of the literature clearly shows, stimulated great interest in liver fat, in the effect of large accumulations of fat on hepatic function, and in the part played by other dietary factors in the protection of the liver.

The very general and broad title assigned to us here has made us somewhat uncertain about what is desired. The topics to be dealt with by our learned colleagues on this comprehensive program leave us but little in the way of definite responsibility, and we are therefore quite free to discuss certain general aspects of our subject.

We are all familiar with the fact that choline, betaine, methionine and, under some conditions, vitamin B₁₂ and inositol, protect rats in varying degrees against the development of fatty livers. In the absence of choline from the diet, many species of animals have been found to develop this abnormality.

Hartroft's presentation (page 633): In some species, such as the guinea pig it is very difficult to produce an abnormal deposition of fat in the liver by feeding diets low in choline.¹³ We must always keep in mind that interference with the function of hepatic cells by means other than deficiency of the lipotropes may lead to fatty livers. Fatty livers of guinea pigs on scorbutic diets¹⁴ are probably not attributable to a lack of dietary choline.

Similar renal lesions have been reported in young pigs¹⁵ and calves¹⁶. The failure to produce this kidney lesion in young mice and puppies, both of which develop fatty livers readily enough, reveals a problem which may possibly require extended study. A comprehensive investigation and comparison of the relevant enzyme systems will undoubtedly help. An interesting sequel to the renal lesion in rats is the appearance in later life of hypertension.¹⁷ This has not yet been reported in other species. You are also familiar with the failure of baby chicks¹⁸ turkey poults¹⁹ and ducklings^{20, 21} to grow and their tendency to develop slipped tendon disease on choline-deficient diets. In our early studies on the effects of choline in preventing and curing the pathological changes in the diabetic dog we mentioned "areas of beginning fibrosis" which did not appear to be as favorably affected by choline as the fatty changes.²²

but we did not observe cirrhosis until after the work of Chaikoff and Connor²² on dogs and György and Goldblatt²³ on rats. The role of choline deficiency in the production of fatty livers and cirrhosis in man is less clear cut. The crucial experiment to determine the lipotropic activity of choline in man has not yet been performed. The nearest approach is that of Phillips, Gabuzda, and Davidson.²⁴

By these brief references to the effects of choline and its precursors in various species, we have been leading up to reconsideration of the word "lipotropic." What should be included under this term? The word was introduced to describe substances which decrease the rate of deposition and accelerate the rate of removal of excess liver fat and was so defined by Best, Huntsman, and Ridout.²⁵ While the word *dietary* was not specifically mentioned, it was obvious from the context that the active principle under discussion was a component of the diet. The word lipotropic has been carelessly used, but some of the misuse is perhaps due to real difficulties in deciding on the proper scope of the term. There is, of course, no justification whatever for many of the misapplications, e.g., a change in lipid content of a fat laden pericardium on the cornea or in the amount of depot fat in obese human subjects or in animals. The term lipotropic is not applicable to changes in lipid concentration in the blood or generally in the body. The word does not apply to increases in liver fat, which at least one writer has suggested. The original definition stated that the term refers to decreases in fat deposition and is applied to changes in the liver. At one time, however, we²⁶ suggested an extension of this to include the other established effects of choline and its precursors, *viz.*, prevention of renal lesions and of cirrhosis. Whether this proposal was wise has since been debated many times in our laboratory. Some of these changes may include effects involving fat deposition *e.g.*, in the kidney²⁷ or perhaps in the walls of blood vessels. If it is firmly established that the primary, or a highly significant part of the process initiated by choline in organs other than the liver is essentially similar to that in the hepatic cells *i.e.* involving the prevention of excess fattiness. The term lipotropic may then be extended to include these other lesions.

Let us compare the antiperiostic action of choline and its effect in preventing the renal hemorrhagic syndrome. We can find no justification for calling the antiperiostic effect lipotropic, but if prevention of deposition of excess fat in the proximal renal tubules is the essential process, this protection of the kidney may be considered a lipotropic phenomenon.

On the other hand, although the growth effect of choline, which can be clearly demonstrated under certain conditions, is mediated through biochemical processes intimately interrelated with lipotropic action, this action should be designated by a term other than lipotropic.

We should stress here that a substance may exert a lipotropic effect without being a lipotropic agent. It might increase the rate of formation of, or potentiate in some way the action of, a lipotropic agent.

The role of antibiotics such as aureomycin in lipotropic phenomena is not too clear. Doctor György will discuss these in detail. We feel that they have

not qualified as lipotropic agents although they do modify the requirement for choline and methionine. Thiamine, calcium, vitamin A or any other dietary essential, in whose absence the food intake and rate of growth are affected, may also affect the choline requirement. We cannot call all the essential food factors antilipotropic agents. The lipotropic effect of the antibiotics cannot be due to change in food intake as Gyorgy has shown, but there is still a possibility that a change in absorption may play a role. The consideration of the antibiotics later in this monograph is of pertinent interest.

To continue our discussion of the term lipotropic, since a fatty liver is always a forerunner of the cirrhosis of choline deficiency, one can properly call the *preventive* effect of choline a lipotropic effect. Can one say, however, that the curative action of choline on a fatty cirrhotic liver is partly or wholly lipotropic? It would appear certain that a part of the reticulum or fibrotic tissue actually disappears as an end result of choline action, but it can not be stated at present whether this result is secondary to an effect on fat to the regeneration of hepatic cells which the disappearance of fat may make possible or to a direct attack on the fibrous tissue.

Another interesting question is raised by the finding¹ that, under certain conditions large accumulations of fat may persist for long periods in the liver without the appearance of fibrosis. In our own experience this has been observed in experiments in which vitamin B₁₂ prevented the development of cirrhosis. This effect of vitamin B₁₂ was first reported from Gyorgy's laboratory, but the details of this particular aspect of their studies have not yet been published. Doctor Drill will be discussing the role of vitamin B₁₂ in lipotropic phenomena. We should like to mention however, our recent finding that rats may be maintained with vitamin B₁₂ on low choline diets (9 per cent protein and very low in methionine) for 16 months without cirrhosis but with

Gyorgy's³¹ original report. This raises the question: does vitamin B₁₂ specifically prevent the cirrhosis, or does it act by increasing the biosynthesis of

be that vitamin B₁₂ acts by increasing the rate of turnover rather than merely reducing the amount of liver fat?

In confirmation of Salmon, Copeland and their colleagues^{22, 23, 24} we have seen a dramatic effect of vitamin B₁₂ on the renal lesions in young rats yet in others on similar diets with a lower 'lipotropic background' no beneficial effect whatever from B₁₂ was observed. Similarly, in older rats others have demonstrated that by alteration in the dietary background one may either reveal or fail to elicit the lipotropic effect of vitamin B₁₂.^{21, 22, 23, 24}

In attempting to account for these observations we have all been forced to recognize the importance of several nutritional dilemmas. The first of these has been appreciated for some time and will be discussed more fully later,

namely, that the

The second diet

the relationship

It became apparent quite early that methionine and choline are essential, since it concerns

diet of a rat or dog affects

systematic study of pro

published In several

and a few crude protein

studies of

deficiency

in every

of the ut

from chol

, volume, mositol, and vitamin B₁₂ offers no serious problem, but

to supply the essential amino acids without choline precursors is a problem

of the highest order While it is true that no proteins free from methionine

are known that are readily available in quantity, the major difficulty

nutritional dilemma rather than the question of

contains a labile methionine

one would like to eliminate

however, that complete

to loss of appetite is known also that when the caloric intake is low, even

severely hypolipotropic diets do not produce fatty livers Thus a diet low in

methionine seems more suitable for lipotropic studies than one completely free

from it

Two chemical procedures are available for eliminating methionine from pro

teins Welch¹¹ hydrolyzed casein with hydriodic acid to demethylate the

methionine After removal of most of the hydriodic acid by evaporation

in vacuo and of the rest with silver carbonate, the remaining amino acids were

obtained for feeding by evaporation of the solution The second chemical

method of obtaining a diet free from methionine, devised by Toennies¹² depends

upon the fact that hydrogen peroxide converts methionine, even in the intact

protein, to the sulphone which is inert nutritionally

To prepare diets low in methionine one may utilize the proteins of peanuts,

peas, or soybeans The proteins may be isolated from these seeds and fed

with other pure chemical substances in synthetic diets or the meals themselves

may be extracted with warm 70 per cent alcohol to remove choline and betaine

and, combined with minerals vitamins fats and carbohydrates to produce

hypolipotropic diets TABLES 1 and 2 show a few of the protein mixtures which

we have used in our laboratory to produce hypolipotropic diets causing fatty

livers, hemorrhagic kidneys or cirrhosis

To each of these protein mixtures the same amounts of minerals vitamins

fats and carbohydrates were added to produce choline-free diets with essen

tially the same total protein content and the same total methionine content

Yet Diet 2 proved as we anticipated to be more severely hypolipotropic than

did Diet 1 The first diet, although it contains 20 per cent of protein is in

adequate with respect to tryptophane threonine and phenylalanine Rats

consuming Diet 1 barely grow at all The much better assortment of

TABLE 1

Diet 1		Diet 2	
Casein	8%	Casein	6%
Gelatin	12	Peanut meal (Alc ext d)	30
Total protein	20	Total protein	21
Total methionine	360 mg	Total methionine (Copeland & Salmon ⁴¹)	360 mg

amino acids in Diet 2 permits good growth. But when growth is improved the choline requirement is increased and, at the same time, the amount of dietary methionine available for lipotropic purposes is diminished. Thus the second diet produces fatty livers of greater severity than does Diet 1 and produces them more rapidly. The increased rate of production and greater severity of the lesions caused by Diet 2 is seen most dramatically in the development of hemorrhagic kidney lesions in weanling rats.

TABLE 2 presents the composition of the protein moiety of several other diets that have been used in our laboratory when still more severely hypolipotropic conditions are desired.

The last diet shown in TABLE 2 contains less than one third the quantity of methionine present in the widely used and severely hypolipotropic mixture of 30 per cent peanut meal (extracted with alcohol) and 6 per cent casein suggested by Copeland and Salmon⁴¹. Diet 6 causes very severe signs of choline deficiency.

been used by many workers, is not nearly as hypolipotropic as one containing 20 per cent arachin or as one containing 12 per cent arachin with 2 per cent fibrin or 8 per cent peanut meal plus 8 per cent of soybean protein. Yet all

many others were slow to appreciate

When one attempts to compare the curative properties of lipotropic agents and proteins in experimental or clinical cirrhosis, one really needs at least four groups of experimental animals. The characteristic features of the four dietary regimens to be compared are shown in TABLE 3.

A protein mixture for the first diet (A) might be 6 per cent alcohol extracted peanut meal plus 6 per cent soybean protein. This contains 9 per cent of

on 50 per cent of alcohol extracted peanut meal plus 5 per cent soybean protein (total protein 30 per cent, methionine 350 mg). To produce Diet D one might add 150 mgm DL methionine (making the total methionine about 500 mgm,

Best et al.: The Lipotropic Factors

651

TABLE 2

Diet 3		Diet 5	
Arachin	12%	Arachin	12%
Gelatin	6	Fibrin	2
Casein	3	Casein	1
Fibrin	1		
Total protein	22%	Total protein	15%
Total methionine	250 mg	Total methionine	150 mg
Diet 4		Diet 6	
Peanut meal (Alc ext'd)	12%	Peanut meal (Alc ext'd)	6%
Soybean protein	12	Soybean protein	6
Total protein	18%	Total protein	9%
Total methionine	192 mg	Total methionine	96 mg

TABLE 3

A	C
Low protein	High protein
Hypolipotropic	Hypolipotropic
B	D
Low protein	High protein
Lipotropic	Lipotropic

as in Diet B) and 0.35 per cent choline chloride. Such a diet would ensure lipotropic adequacy. Omission of any one of the groups makes a valid comparison of the effect of protein *per se* with that of lipotropic agents, in the treatment of cirrhosis, impossible. No study of this type has yet been published. Ideally, four more groups should be included, as shown in TABLE 4. These would permit us to separate out even more clearly the therapeutic factors of greatest influence. By low quality proteins, we mean in this connection those poor in methionine. A number of experiments along such lines were performed in our laboratory several years ago. Others are currently in progress and more are planned. In practically all of these we have tried to use diets in which all the essential amino acids were present in amounts sufficient to permit maintenance and repair of the tissues. We now look askance at curative experiments conducted with diets extremely low in protein since these obviously could not provide the building blocks needed for repair. Addition of protein to such a basal diet supplies the building blocks which choline alone cannot obviously furnish when good protein, which contains methionine, is fed, both the building blocks and a source of choline are supplied simultaneously. From the therapeutic point of view this is ideal, but scientifically it is of no value in elucidating the fundamental mechanisms involved. Protein building blocks without choline or its precursors have always failed to give protection and, in cirrhosis caused by carbon tetrachloride, were shown to possess no curative value.

TABLE 4

E	G
High protein (low quality) Extra methionine (DL)* No added Choline	Better quality protein No free methionine added No choline added
F	H
High protein (low quality) No extra methionine Choline chloride	Better quality protein Choline chloride

* As amount necessary to make the total methionine in D, E, F and G equal

We attempt to resolve the first dilemma mentioned earlier by providing barely enough methionine to meet the requirements for growth and make the unsupported and, we believe, unwarranted assumption that none would be used for synthesis of choline. This dilemma is further complicated by the well established increased need for lipotropes when the growth rate is augmented by adding methionine. We should attempt to meet the more complicated second dilemma by using just enough methionine to eliminate the first dilemma and just sufficient vitamin B₁₂ to satisfy other than lipotropic requirements, i.e., trusting that none of either the vitamin B₁₂ or methionine is "misappropriated" for lipotropic purposes. The experimental evidence suggests that this ideal also may be impossible of attainment.

and part of the second, would be avoided. Failing this, we must try to find an otherwise innocuous chemical that blocks the enzymes transferring the methyl group of methionine to form choline. Even more helpful would be the discovery of a substance that would selectively block methylation of aminoethyl alcohol. This would resolve our present dilemmas and permit a breathing space until some new complications are revealed.

References

1. BEST, C. H., J. M. HERSHEY & M. E. HUNTSMAN. 1932. *Am J Physiol* 101: 7P.
2. BEST, C. H. & M. E. HUNTSMAN. 1932. *J Physiol* 75: 405.
3. BEST, C. H., M. E. HUNTSMAN & D. M. SOLANDT. 1932. *Trans Roy Soc Canada* 26(5): 175.
4. BEST, C. H., C. C. F. & M. E. HUNTSMAN. 1932. *J Biol Chem* 104: 1109.
5. HANDLER, P. 1949. *Proc Soc Exptl Biol Med* 70: 187.
6. J. Nutrition.
7. JOHNSON, B. C. & M. F. JAMES. 1948. *J Nutrition* 36: 339.
8. BERNARD, R. & J. M. DEWEES. 1949. *Can J Res (E)* 27: 281.
9. HANDLER, P. 1949. *Proc Soc Exptl Biol Med* 70: 70.
10. SPELLBERG, M. & R. W. KEETON. 1939. *Proc Soc Exptl Biol Med* 41: 570.

Best et al The Lipotropic Factors

653

- 15 GRIFFITH W H & V J WADE 1939 J Biol Chem 131 567
- 16 NEUMANN A L J L. KRIDER, W F JAMES & B C. JOHNSON 1949 J Nutrition 38 195
- 17 HARTROFT W S & C. H. BEST 1949 Brit. Med J 1 423
- 18 JUKES T H 1941 Proc Soc Exptl Biol Med 46 155
- 19 JUKES T H 1940 J Biol Chem 134 789
- 20 ROOS A D M HEGSTED & F J STARR 1946 J Nutrition 32 473
- 21 BERNARD R. & J M DEMERS 1934 Rev Can. Biol 7 174
- 22 MACLEAN D L & C L CONNOR 1940 Brit J Exptl Path. 15 193
- 23 CHAIKOFF I L & C H BEST 1939 J Exptl Med 70 185
- 24 GYORGY I & H GOLDBLATT 1939 J Exptl Med 70 185
- 25 PHILLIPS G B G J GABOZDA & C S DAVIDSON 1952 J Clin Invest 31 351
- 26 BEST C H M E HUNTSMAN & J H RIDOUT 1935 Nature 135 821
- 27 SELLERS E A C C LUCAS & C H BEST 1948 Brit Med J 1 1061
- 28 HARTROFT W S & C H BEST 1947 Science 105 315
- 29 HANDLER P & I A DUBIN 1946 J Nutr on 31 141
- 30 Unpublished data
- 31 GYORGY P 1951 Liver Injury Trans Ninth Conf (1950) Josiah Macy Jr Foundat on. Ed F W Hoffbauer New York 206
- 32 SCHAEFER A E W D SALMON & D R STRENGTH 1949 Proc Soc Exptl Biol Med 71 193
- 33 SCHAEFER, A E. W D SALMON D R STRENGTH & D H COPELAND 1950 J Nutrition 40 95
- 34 STRENGTH D R A E SCHAEFER & W D SALMON 1951 J Nutrition 45 329
- 35 GYORGY P & C S ROSE 1930 Proc Soc Exptl Biol Med 73 372
- 36 DRILL V A & H M MCCORMICK 1949 Proc Soc Exptl Biol Med 72 183
- 37 MCCORMICK H M & V A DRILL 1950 Proc Soc Exptl Biol Med 74 66
- 38 BENNETT M A J JOSEPHSON & F E HALPERN 1951 J Biol Chem 193 285
- 39 WELCH A D 1941 J Biol Chem 137 173
- 40 TOENNIES G 1942 Federation Proc 1 138
- 41 COPELAND D H & W D SALMON 1946 Am J Path 22 1059

LIPOTROPIC EFFECTS OF VITAMIN B₁₂ AND OTHER FACTORS

By Victor A. Drill

Wayne University College of Medicine, Detroit, Mich

Our interest in lipotropic substances other than methionine, casein, or choline followed the publication of studies of Gillman and Gillman on fatty livers in children with pellagra¹. By means of serial biopsies of the liver, they were able to demonstrate a marked lipotropic of hog stomach (Ventriculin). They also reported a partial lipotropic effect of liver extract and a minimal effect of brewer's yeast.

A survey of the literature showed a lack of knowledge concerning the lipotropic effect of such supplements in experimentally induced liver injury. Hog stomach had not received any experimental study. With regard to liver extract, an early study by Rhoads and Miller² reported that liver extract only partially restored to normal the abnormal liver function produced in dogs by a black tongue diet. In 1942, Gyorgy and Goldblatt³ studied the effectiveness of a liver concentrate in preventing dietary cirrhosis in rats and concluded that the "liver extract was completely ineffective in preventing hepatic injury". The liver concentrate used in this later study was not commercial liver extract but the fraction of an aqueous liver extract soluble in alcohol. Yeast had received somewhat greater study but the results were conflicting^{4, 5}. Our first study, therefore, concerned the possible lipotropic effect of hog stomach liver extract, and yeast.

The fatty livers were induced by a normal protein—high fat diet or a low protein—low fat diet (TABLE 1). Each rat also received a daily oral supply of the B vitamins as follows:

thiamin	20 µg
riboflavin	25 µg
pyridoxine	20 µg
calcium pantothenate	100 µg

Vitamins A and D were supplied by adding 5 drops of oleum percomorphum per kilo of diet.

In the first study the following supplements were administered to rats receiving the high fat or the low protein diet: (a) crude liver extract (Lilly, 1 USP unit per cc), 1 cc subcutaneously, 3 times a week, (b) brewer's yeast, 0.25 gm per rat per day, mixed with the diet, (c) hog stomach (Ventriculin), 0.5 gm per rat per day, mixed with the diet. Control groups of rats received the following: (a) choline chloride 40 mgm per day subcutaneously, (b) DL-methionine 40 mgm per day subcutaneously. One group receiving the high fat diet received 8 per cent additional casein in the diet, the content of corn starch being decreased by 8 per cent. Similarly, with the low protein diet, one group received an additional 0.5 per cent casein in the diet to control the small amount of protein added by the various supplements.

Untreated rats developed a marked fatty change, followed, depending on the length of the study, by fibrosis. The complete effectiveness of the choline

Drill Lipotropic Effects of Vitamin B₁₂

655

TABLE 1
EXPERIMENTAL DIETS

	Control diet %	Diet 1 %	Diet 2 %
Use normal rat starch	16	16	6
rose	6	51	6
t mixture	60	30	70
	15	—	15
	3	3	3
	100	100	100

TABLE 2
EFFECT OF VARIOUS SUPPLEMENTS ON FATTY LIVER

Group	No. animals	Liver fat gm %
Exp 1 200 days (diet 16% protein 51% fat)		
High fat	13	20.5 ± 1.72
Ventricul n	10	21.7 ± 1.38
Methionine	10	17.8 ± 2.40
Casein 24%	9	15.4 ± 1.11
Yeast	10	11.2 ± 0.92
Liver extract	10	8.8 ± 0.65
Choline	10	7.3 ± 0.64
Exp 2 200 days (diet 6% protein 6% fat)		
High fat	4	23.1 ± 2.94
Ventricul n	4	21.4 ± 7.26
Methionine	4	17.8 ± 8.20
Yeast	4	7.8 ± 0.84
Liver extract	4	7.2 ± 0.56
Choline	3	7.2 ± 1.03

in preventing these lesions served as a base line to compare the lipotropic effects of the other supplements. Microscopic examination or fat analyses indicated that the liver extract completely prevented the fatty changes in the liver of rats fed a high fat diet. Supplements of ventriculin or methionine were without effect. Casein was only slightly effective and yeast was partially effective (TABLE 2). Liver extract was equally potent in preventing fatty livers in animals fed the low protein diet. With this diet the yeast supplement also offered complete protection whereas ventriculin and methionine were without lipotropic activity.

Food intake was measured in each study and the lipotropic activity of the supplements was not related to changes in caloric or protein intake. There was also no correlation between lipotropic activity and gain in body weight. In further studies it was noted that oral liver extract 1 cc three times a week is slightly less lipotropic than the same dose given by injection.

The lipotropic activity of these supplements cannot be explained on the basis

TABLE 3

LIPOTROPIC EFFECT OF DIFFERENT VITAMIN B₁₂ CONCENTRATES PREPARED FROM LIVER

No. animals	Diet	Dose of conc. in terms of μ g. of B ₁₂	Liver fat gm. %
Exp. 1, 66 days			
4	Normal	—	5.6 \pm 1.08
8	High fat	—	24.6 \pm 0.59
10	High fat	1.0	7.8 \pm 0.89
Exp. 2, 50 days			
7	Normal	—	6.3 \pm 0.82
7	High fat	—	15.3 \pm 2.01
5	High fat + #4	1.0	13.1 \pm 1.01
5	High fat + #4	4.0	13.8 \pm 1.27
5	High fat + #2	1.0	11.3 \pm 1.35
5	High fat + #2	4.0	10.4 \pm 0.95
5	High fat + #3	1.0	7.4 \pm 0.88
5	High fat + #3	4.0	7.8 \pm 0.62

of their choline content. Of all of the supplements, Ventriculin supplied the largest amount of choline, namely, 1.8 to 2.1 mgm. per day. Although the liver extract completely prevented the fatty changes, it supplied approximately

for lipotropic effects.

We then observed that certain vitamin B₁₂ concentrates prepared from liver were also lipotropic.⁹ The activity of such extracts, however, varies quite considerably.¹⁰ The extracts were injected three times a week in terms of their vitamin B₁₂ activity and the total amount of extract administered was usually 0.5 cc. three times a week. The lipotropic potency of such extract can vary from inactivity, partial effect to complete prevention of the fatty changes (TABLE 3). These vitamin B₁₂ concentrates supplied only 0.2 mg. of choline and 0.08 mg. of methionine per day, an amount too small to explain their lipotropic activity. One concentrate of vitamin B₁₂ prepared from streptomycin broth was studied but proved to be too toxic to clearly evaluate lipotropic activity. A growth promoting factor which has been highly purified

higher dose of the extract, however, was somewhat toxic.¹¹

In view of lipotropic effect of liver extract and vitamin B₁₂ concentrate and the fact that their activity could not be explained on the basis of choline content, further studies were designed using the high fat diet, in which small amounts of supplements were administered in different combinations to rats.

TABLE 4
EFFECT OF VITAMIN B₁₂ CONCENTRATE ON RATS FED A HIGH FAT DIET FOR 30 DAYS

Animals	Diet	B ₁₂ conc. (3 X week)	Liver fat %
5	Low fat	none	7.02 ± 0.72
5	High fat	none	23.9 ± 1.40
5	High fat	0.3 cc	18.4 ± 0.24
3*	High fat	0.8 cc	11.9 ± 4.39

* Two animals died on the 12th and 26th days

TABLE 5
EFFECT OF SMALL DOSES OF SINGLE SUPPLEMENTS ON LIVER FAT

No. animals per group	Normal diet	High fat diet untreated	High fat diet treated	Supplement (3 X week)	Dose	Days of exp.
10	4.8 ± 1.20	17.5 ± 1.49	14.1 ± 1.77	B ₁₂	1 µg	30
8-10	6.8 ± 0.31	28.8 ± 1.03	27.3 ± 1.12	B ₁₂	2.5 µg*	64
8-10	8.4 ± 1.05	26.7 ± 1.83	26.2 ± 2.54	B ₁₂	10 µg	30
			24.6 ± 0.83	B ₁	40 µg	30
6	—	18.9 ± 1.98	18.3 ± 2.54	B _{12b}	10 µg	64
10	4.8 ± 1.20	17.5 ± 1.49	23.4 ± 1.83	Choline	10 mg	30
			26.1 ± 2.38	Choline	20 mg	30
6	—	18.9 ± 1.98	16.4 ± 1.27	Folic acid	2.5 mg	64
			21.0 ± 3.81	Folic acid	25.0 mg	NT
			16.3 ± 2.19	Citrov. fact.	2,500 units	64
			16.8 ± 1.67	Citrov. fact.	25,000 units	64
6	6.3 ± 0.29	24.9 ± 1.14	20.7 ± 1.85	Inositol	20 mg	33
			18.9 ± 2.38	Inositol	40 mg	33
8	5.8 ± 0.27	19.2 ± 1.67	16.4 ± 1.72	Aureomycin	50 mg	31

* 5 times per week

receiving a high fat diet. The amounts of supplements administered were similar to those present either in liver extract or vitamin B₁₂ concentrate. Although small amounts of each material present in liver extract may be ineffective by itself, it may, when given in combination with other materials, demonstrate lipotropic activity. The single supplements studied were B₁₂, B_{12b}, choline, folic acid, citrovorum factor, inositol, and aureomycin. Each of these alone (TABLE 4) in the doses used were ineffective in preventing fatty changes in the liver of animals fed a high fat diet.* Gyorgy and Rose also reported that crystalline vitamin B₁₂ was without lipotropic effect in animals fed a high fat diet. They did, however, obtain a partial lipotropic effect of

of retinal hemorrhagic necrosis in rats. Our attempts to demonstrate a sparing effect of B₁₂ on choline in the lipotropic studies were unsuccessful (TABLE 5). The doses of choline were graded and were greater than the amount of choline present in liver extract or vitamin B₁₂ concentrate.* Thus the lipotropic of liver extract cannot be explained by a sparing effect of vitamin B₁₂ on the amount of choline present. A combination of choline and vitamin

TABLE 6

EFFECT OF CHOLINE, VITAMIN B₁₂, AND VITAMIN B_{12b} ON LIVER FAT OF RATS FED A HIGH FAT DIET

No. animals	Diet	Choline mgm	B ₁₂ μg	B _{12b} μg	Liver fat gm %
Experiment 1, 30 days					
10	Normal	—	—	—	4.8 ± 1.20
10	High fat	—	—	—	17.5 ± 1.49
9	High fat	0.5	1.0	—	19.0 ± 1.53
10	High fat	1.0	1.0	—	23.5 ± 1.26
10	High fat	2.0	1.0	—	18.9 ± 2.24
Experiment 2, 64 days					
6	High fat	—	—	—	18.9 ± 1.98
5	High fat	2.0	—	1.0	20.1 ± 2.27
Experiment 3, 72 days					
4	Normal	—	—	—	5.2 ± 0.11
5	High fat	—	—	—	23.7 ± 1.11
6	High fat	4	2	—	19.2 ± 0.11

TABLE 7

LIPOTROPIC EFFECT OF CHOLINE, VITAMIN B₁₂ AND B_{12b} IN COMBINATION WITH FOLIC ACID AND CITROVORUM FACTOR

No. animals	Diet	Choline mg	B ₁₂ μg	B _{12b} μg	Folic acid μg	Citrov. factor units	Liver fat gm %
Experiment 1, 64 days							
6	High fat	—	—	—	—	—	18.9 ± 1.1
6	High fat	2	1	—	—	2,500	16.9 ± 1.1
6	High fat	2	1	—	—	25,000	11.4 ± 1.1
6	High fat	2	1	—	2.5	—	12.1 ± 0.1
6	High fat	2	1	—	25.0	—	11.4 ± 0.1
5	High fat	2	—	1	—	25,000	10.7 ± 1.1
5	High fat	2	—	1	25.0	—	10.5 ± 1.1
Experiment 2, 72 days							
4	Normal	—	—	—	—	—	5.2 ± 0.11
5	High fat	—	—	—	—	—	23.7 ± 1.12
6	High fat	2	1	—	2.5	—	14.4 ± 1.12
7	High fat	4	2	—	2.5	—	14.3 ± 0.78

B_{12b} was also ineffective¹⁴ and vitamin B₁₂ does not exert a sparing effect on methionine in lipotropic studies¹⁶ (TABLE 6)

During the past two years studies were also performed with folic acid or citrovorum factor in combination with choline and vitamin B₁₂. In the previous studies the liver extract supplied an average 1.2 μg. and the vitamin B₁₂ concentrate an average of 0.03 μg. of folic acid per day. The addition of

TABLE 8
LIPOTROPIC EFFECT OF CHOLINE, VITAMIN B₁₂, FOLIC ACID AND INOSITOL

No. animals	Diet	Choline mg/100	B ₁₂ μg	Folic acid μg	Inositol mg	Liver fat gm %
Experiment 1 92 Days						
12	Normal	—	—	—	—	6.8 ± 0.49
12	High fat	—	—	—	—	18.7 ± 1.39
9	High fat	0.5	—	1	0.5	20.1 ± 0.58
10	High fat	1.0	—	2	1.0	17.7 ± 2.48
Experiment 2 33 Days						
6	Normal	—	—	—	—	6.3 ± 0.29
6	High fat	—	—	—	—	24.9 ± 1.14
6	High fat	2.0	—	2.5	2.0	19.5 ± 1.10
6	High fat	2.0	—	10.0	2.0	17.3 ± 3.25
6	High fat	2.0	1.0	2.5	2.0	14.9 ± 1.62
5	High fat	2.0	1.0	10.0	2.0	16.8 ± 2.30
6	High fat	2.0	1.0	10.0	4.0	15.8 ± 2.10

TABLE 9
LIPOTROPIC EFFECT OF SMALL AMOUNTS OF VARIOUS SUBSTANCES

No. animals	Diet	Choline mg	B ₁₂ μg	Folic acid μg	Inositol mg	Ascomycin mg	Liver fat gm %
6	Normal	—	—	—	—	—	5.8 ± 0.27
8	High fat	—	—	—	—	—	19.2 ± 1.67
8	High fat	2	—	10	4	5	17.6 ± 2.05
9	High fat	2	1	10	—	5	11.0 ± 0.83
8	High fat	2	1	10	4	5	12.6 ± 1.71

folic acid to the choline and the B₁₂ produced a partial lipotropic effect.¹¹ This effect of folic acid could also be obtained by administering 25,000 units of citrovorum factor with the B₁₂ and choline, and vitamin B_{12b} was also effective in replacing vitamin B₁₂ in such a combination of supplements (TABLE 7). These three supplements were not as effective as liver extract, for in each study the liver fat was only returned about half way to normal.

In the earlier studies, the liver extract supplied 0.9 mg of inositol per injec-

Discussion

The establishment in 1948 of the lipotropic activity of liver extract in rats fed a high fat diet demonstrated that factors other than choline can markedly influence the fat content of the liver.⁸ The lipotropic effects of vitamin B₁₂ concentrates prepared from liver are also not explainable on the basis of choline content.^{9, 10} That vitamin B₁₂ was important was demonstrated by Schaefer

and co workers who reported a sparing action of this vitamin on the choline requirement of rats and chicks¹⁷ Vitamin B₁₂ and other factors may act to alter either the rate of synthesis of labile methyl groups or the process of transmethylation, but the exact mechanism of such effects is not known It is now known that the methyl groups of choline and methionine can be synthesized

concluded that supplements of vitamin B₁₂, folacin, and homocystine did not prevent the renal injury and death of weanling rats (40-50 gm) in the absence of a dietary source of labile methyl groups²⁶ The variation in results may be due to the difference in requirement among the strains of rats²⁷

Other studies have also shown that vitamin B₁₂ is necessary for the methylation of homocystine to form methionine A diet deficient in vitamin B₁₂ interferes with the ability of the liver to convert betaine and homocystine to methionine or choline and homocystine to methionine²⁸ Jukes, Stokstad and Broquist observed that vitamin B₁₂ was necessary for the methylation of homocystine to obtain growth in chicks fed a methionine deficient diet²⁹ This action of vitamin B₁₂ depends however, on the amount of methyl groups available When weanling rats are fed a diet deficient in choline and methionine it was observed that choline alone will prevent the renal injury but gain in body weight is very slight In the presence of choline Schaefer and Knowles²⁴ observed that supplements of vitamin B₁₂ alone allowed an adequate gain in weight, indicating that the synthesis of methionine from homocystine was facilitated When, however, the amount of methyl groups was limited by a further reduction of choline intake both vitamin B₁₂ and folacin were required to allow adequate growth

A deficiency of folic acid in the chicken has also been reported to interfere with the methylation of homocystine³¹ and still other studies show the need for both folic acid and vitamin B₁₂ Schaefer and Knowles²⁴ showed that supplementations of a basal diet with homocystine, betaine, and aminoethanol failed to support growth or prevent renal injury With 0.41 per cent betaine and 0.05 per cent aminoethanol in the diet the addition of vitamin B₁₂ prevented renal injury and permitted good growth With a lesser amount of betaine and aminoethanol in the diet supplements of both vitamin B₁₂ and folic acid were required to prevent renal injury and allow growth Such weanling rats fed deficient diets also develop fatty livers The addition of vitamin B₁₂ and folic acid suppressed the accumulation of fat in the liver of rats fed subprotective levels of choline³²

Of particular interest is the relation of betaine aldehyde or betaine to choline oxidase and transmethylation reactions. Betaine is a methyl donor for the synthesis of choline and other methyl groups. It has been reported that betaine inhibits choline oxidase activity and that folic acid is required for the synthesis of betaine. The addition of betaine to a diet deficient in choline and vitamin B₁₂ prevents renal injury and allows growth in rats. The addition of betaine to a diet deficient in choline and folic acid also prevents renal injury and allows growth in rats. The addition of betaine to a diet deficient in choline and both vitamin B₁₂ and folic acid also prevents renal injury and allows growth in rats.

plays a part in the conversion of choline or betaine aldehyde to the actual methyl donor, betaine, and its transmethylation to homocystine. Stekol *et al.* have found that a deficiency of folic acid in the adult rat reduces the capacity to utilize glycine or serine for the production of choline. During vitamin B₁₂

extract, the various substances present in liver extract were administered alone and in various combinations. The amount of each supplement was equal to or slightly greater than that present in liver extract or vitamin B₁₂ concentrates. The small amounts of choline or vitamin B₁₂ present in such liver extract were

to enable a sparing effect of vitamin B₁₂ to be demonstrated. The further addition of folic acid to a combination of vitamin B₁₂ and choline produced a partial reduction in liver fat. The amount of folic acid used was about ten times that present in liver extract. The folic acid was not effective alone and had to be used in combination with choline and vitamin B₁₂. The further addition of inositol or aureomycin failed to influence further the deposition of fat in the liver. The best results, therefore, have been obtained with a mixture of these three supplements and there must be other factors in the liver extract that can influence the synthesis of labile methyl groups.

With regard to other diets and results, Gyorgy and co-workers reported that liver extract may enhance the effect of casein or of methionine in treating experimental cirrhosis³⁹ and that vitamin B₁₂ does not affect the production of necrosis with a necrogenic diet.¹⁴ Using normal diets, Popper⁴⁰ has also found that vitamin B₁₂ concentrate will inhibit the fatty changes and depletion of

cessation of carbon tetrachloride, however, brought about a more rapid reversal of the hepatic damage. Hove has also demonstrated that vitamin E or vitamin B₁₂ can increase the survival, under certain dietary conditions, of rats receiving toxic doses of CCl₄.⁴¹ The choline oxidase activity of the liver was not influenced by vitamin E or by CCl₄.⁴²

Aureomycin may exert a sparing effect on vitamin B₁₂, as measured by growth response in rats and chickens,⁴³⁻⁴⁵ and Gyorgy has reported that aureomycin can exert a lipotropic effect and an antinecrotic effect in experimental animals.⁴⁶⁻⁴⁸ It is generally assumed that aureomycin given orally acts to alter the bacterial floor of the gut. This may occur by a decrease in the number of *E. coli* in the gut and a simultaneous increase in *Bacillus megatherium*, an organism that can produce large quantities of vitamin B₁₂.⁴⁹ In our own studies, the administration of aureomycin by injection did not improve the partial lipotropic effect of folic acid, vitamin B₁₂, and choline.

The problem becomes more complicated by interrelationship between cortisone, vitamin B₁₂, and folic acid. Cortisone will increase the excretion of vitamin B₁₂ in pigs fed a vitamin B₁₂ deficient diet and thereby reduce body growth and survival¹⁰. Vitamin B₁₂ or aureomycin can largely counteract the growth inhibiting effects of cortisone in rats¹¹. Cortisone can also replace pteroylglutamic acid and citrovorum factor for the growth of *Streptococcus faecalis* and *Leuconostoc citrovorum* and can increase the growth inhibiting effects of aminopterin on *Leuconostoc citrovorum*¹².

Summary

The lipotropic effect of liver extract or certain vitamin B₁₂ concentrates prepared from liver cannot be accounted for by their content of choline. Using various supplements in the amounts present in these extracts it was found that a combination of choline, vitamin B₁₂, and folic acid produced a partial lowering of liver fat in rats fed a high fat diet. In the absence of any one supplement from this mixture liver fat was not even partially reduced. Vitamin B₁₂ could be replaced by vitamin B_{12b} and citrovorum factor could replace folic acid. The further addition of inositol and aureomycin was without added effect in lowering liver fat.

Investigators have shown a certain relationship of vitamin B₁₂ and folic acid to the synthesis of labile methyl groups and to their use in the transmethylation procedures as judged by the growth of animals or the prevention of renal injury. It is evident that when diets are primarily designed to produce fatty changes in the liver, a factor or factors other than choline, vitamin B₁₂, and folic acid are required to allow maintenance of a normal liver fat.

References

1. GILLMAN T & J GILLMAN. 1945. Arch. Internal Med. 76: 63.
2. RHOADS C P & D K MILLER. 1938. J. Exptl. Med. 67: 463.
3. GEORGY P & H GOLDBLATT. 1942. J. Exptl. Med. 75: 355.
4. WEBSTER G T. 1942. J. Clin. Invest. 21: 385.
5. HINSWORTH H P & L E GLYNN. 1944. Clin. Sci. 11: 93.
6. HALL C A & V A DRILL. 1948. Proc. Soc. Exptl. Biol. Med. 69: 3.
7. DRILL V A & C A HALL. 1950. Am. J. Med. Sci. 219: 197.
8. MCCORMICK H M & V A DRILL. 1950. Proc. Soc. Exptl. Biol. Med. 74: 626.
9. DRILL V A & H M MCCORMICK. 1949. Proc. Soc. Exptl. Biol. Med. 72: 388.
10. DRILL V A & H D LAIRD. Unpublished data.
11. SLOAN M J. 1944. Poultry Sci. 20: 85.
12. NOVACK A F & S M HAUGE. 1948. J. Biol. Chem. 174: 647.
13. ACSTIN F L & C S BORTFF. 1949. Proc. Fourth Conf. on Feeds of the Grain & Oilseeds. 7.
14. LAIRD H D, H M MCCORMICK & V A DRILL. Unpublished data.
15. LAIRD R D & V A DRILL. Unpublished data.
16. GEORGY P & C B ROSE. 1950. Proc. Soc. Exptl. Biol. Med. 73: 372.
17. SCHAEFER A E, W D SALMON & D R STRENGTH. 1949. Proc. Soc. Exptl. Biol. Med. 71: 193.
18. SCHAEFER A F, W D SALMON & D R STRENGTH. 1949. Proc. Soc. Exptl. Biol. Med. 71: 202.
19. GILLIS M B & L C MORRIS. 1949. Poultry Sci. 28: 749.
20. SAKAMI W. 1950. J. Biol. Chem. 187: 369.
21. SAKAMI W & A D WELCH. 1950. J. Biol. Chem. 187: 379.
22. DU VIGNEAUD V, C RESSLER & J H RACHELE. 1950. Science. 112: 267.
23. ARNSTEIN H R V. 1951. Biochem. J. 48: 27.
24. BENNETT M A. 1950. J. Biol. Chem. 187: 751.

- 25 STEAOL J H & K WEEDS 1950 J Biol Chem 186 343
- 26 SCHAEFER A J & J I KNOX 1951 Proc Soc Exptl Biol Med 77 656
- 27 HANLIER P & H H FOLLES JR 1950 Proc Soc Exptl Biol Med 76 567
- 28 HALF O M & A I SCHAEFER 1951 Proc Soc Exptl Biol Med 77 633
- 29 OGINSKY F L 1950 Arch Biochem 26 327
- 30 JULYS T H I R STOKSTAD & H H BROQUIST 1950 Arch Biochem 25 453
- 31 JULYS T H 1952 Federation Proc 11 447
- 32 STRENGTH D R A I SCHAEFER W H SALMON & D H COPELAND 1950 J Nutrition 40 95
- 33 MUNTZ J A 1950 J Biol Chem 182 489
- 34 WILLIAMS J N JR 1951 J Biol Chem 191 123
- 35 DUNNING J S C K KEITH I L DAVIS & P L DAY 1950 Arch Biochem 27 89
- 36 WILLIAMS J N JR 1951 Proc Soc Exptl Biol Med 76 202
- 37 WILLIAMS J N JR 1951 Proc Soc Exptl Biol Med 76 206
- 38 STEAOL J A et al 1952 Federation Proc 11 272
- 39 CARY P & H GOLDBLATT 1949 J Exptl Med 90 73
- 40 LOPPER H D KOCI WESER & I B SZAVO 1949 Proc Soc Exptl Biol Med 71 688
- 41 MUSHETT C A O F R A
- 42 HOVE I L
- 43 HOVE I L
- 44 WHITEHILL " Exptl Biol Med 74 11
- 45 CRAVOTO MENDOZ J H G PONCER & H A WAISMAN 1951 Proc Soc Exptl Biol Med 77 18
- 46 STOKSTAD I L R & T H JULYS 1951 Proc Soc Exptl Biol Med 76 73
- 47 GREGORY I J STOKES JR & H GOLDBLATT 1951 Trans Assoc Am Physicians 64 280
- 48 GREGORY P J STOKES JR W H SMITH & H GOLDBLATT 1950 Am J Med Sci 220 6
- 49 IREIS J C K IJIMI N J SNELL & J A GARIBALDI 1949 Bur Agr Ind Chem Publication No 254 USDA
- 50 WAILSTROM R C & B C JOHNSON 1951 Proc Soc Exptl Biol Med 76 112
- 51 MEITZ J 1952 Proc Soc Exptl Biol Med 81 307
- 52 GAINES D S H P BROQUIST & W L WILLIAMS 1951 Proc Soc Exptl Biol Med 77 247

Discussion of the Paper

D. M. S. A. T.

tration and purification of Factor 3 from natural sources especially from enzymatic casein digests we have found that certain fractions do not protect against dietary necrotic liver degeneration but actually enhance development of the disease. We have accumulated much evidence that sources of methyl groups such as choline and betaine and also substances which are catalytically involved in one carbon unit metabolism such as vitamin B₁₂ and citrovorum factor have such an enhancing effect on the development of liver necrosis (Schwarz Introduction page 617). According to our conception that there is an antagonistic balance between liver necrosis on the one hand and fatty liver and cirrhosis on the other hand we have tested our liver necrosis-accelerating fractions for protective activity against fatty infiltration of the liver. In pre-

LIVER CARCINOMA AND RELATED LESIONS IN CHRONIC CHOLINE DEFICIENCY*

By W. D. Salmon and D. H. Copeland

Department of Internal Husbandry and Nutrition, Agricultural Experiment Station of the Alabama Polytechnic Institute, Auburn, Ala.

In studies of the pathology of chronic choline deficiency in the rat, the development of various types of neoplasms was observed in a significant percentage of experimental animals. Some of these results have been reported by Copeland and Salmon in 1946,¹ Engel, Copeland, and Salmon in 1947,² and Schaefer, Copeland, Salmon, and Hale in 1950.³ Over 500 chronic choline deficient rats have been studied in this laboratory. Since the finding of neoplasms in these animals was most frequent in the liver, it is the purpose of this paper to describe the sequence of lesions culminating in the appearance of liver carcinoma. In another series of experiments, chickens were used to determine whether it would be possible to produce neoplasms by chronic choline deficiency in a species of animal other than the rat. The fatty changes, cirrhosis, and neoplasms of the liver, as well as some kidney changes which developed, are compared to corresponding lesions in the rat.

The general procedure followed and typical choline-deficient diets used in our laboratory have been described in previous publications.¹⁻⁴

Histologic Studies on Livers of Chronic Choline Deficient Rats

Fatty livers and cirrhosis. The livers of rats that died during the first four weeks of the experiments were swollen and quite light in color. Microscopically there was a marked accumulation of fat uniformly involving all lobes of the liver, and apparently all the liver cells. On the other hand sections from the livers of rats that had been on the choline deficient diet for as long as three months showed a zonal distribution of fatty infiltration with marked accumulations of fat in some areas forming peculiar architectural alterations.¹ These are similar to lesions described as fatty cysts by Hartroft and Ridout.⁵ In later stages of the deficiency, the amount of fat appeared to decrease as cirrhosis advanced in extent and severity. Some lobules of the liver contained considerable fat but others seemed to be composed largely of newly regenerated liver cells. Ceroid was present in all cirrhotic livers but varied in amount. Mitotic figures were found in the cirrhotic livers indicating that regeneration of new parenchymal cells was taking place at a rapid rate. There was a pronounced variation in the size of the liver cells. Bile duct proliferation was seen in a majority of the livers.

Neoplasms of liver. In the livers of all the rats that remained on the choline deficient diets for eight months or longer severe nodular cirrhosis was observed. Large regeneration nodules 0.5 to 1 cm. in diameter were common. Tumors

* Published with the approval of the Director of the Alabama Agricultural Experiment Station. This work was supported in part by grants from the American Cancer Society upon the recommendation of the Committee on Growth of the National Research Council from the National Cancer Institute and from the Nutrition Foundation, Inc.

The authors are also indebted to Dr. William V. Hare, Pathology Department, University of Mississippi Medical School for his assistance in interpretation of the microscopic material.

developed in a significant number of cases. The incidence of tumors of the liver in the rat varied in the different experiments from 16.1 to 30 per cent with an average of 23.1 per cent. The tumors varied in size from 0.8 to 3.5 cm. in diameter and were of a different consistency from the regeneration nodules. The appearance of one of the liver tumors is illustrated in FIGURE 1.

2, 3, and 4

variation in

tumor. The

alveolar arrangement and general appearance of the cells in some areas seem to point to a bile duct origin, but in most cases the cell type suggests an origin from liver cord cells.

Histologic studies are being continued on all tumors that developed in chronic choline deficient rats in an attempt to determine which of these tumors are definitely malignant. Special stains were carried out on most malignant or "possibly malignant" lesions. Histologic determination of the malignancy of many of these "tumors" is a matter of judgment. We have attempted to be conservative by grouping many of them under the classification "atypical regeneration nodules."

In many tumor cases, invasion of the mesentery was noted grossly and verified microscopically. In some of these cases, little or no connection was noted

prove that these structures were blood vessels. However, this did not eliminate the possibility of these structures representing lymphatic invasion. The appearance of the tumor nodules in these areas suggests that, as the tumors spread into mesenteric tissue, they incite the production of a limiting fibrous wall. One slide in each of two cases did show definite vein invasion. With the exception of these two cases, the stroma of the tumor blended too well with the fibrous wall for that wall to be a blood vessel. There were proliferating bile ducts at the edge of some of the tumor islands.

An interesting feature in the study of periodic acid stains on sections of the

a large amount of collagen in the cirrhotic, nontumorous portions of the liver, but in the tumor proper, there was a relatively small amount around alveolar structures. In slides prepared by Foot's modification of Bielschowsky's reticulum stain, there is a characteristic alveolar pattern in some parts of the tumor which is typical of epithelial origin.

No fibrils arising from tumor cells were demonstrated by phosphotungstic acid stain. There was some PAS positive material in some of the macrophages in the cirrhotic livers which is probably ceroid.

All the blocks of lung tissue from choline deficient rats with liver tumors are

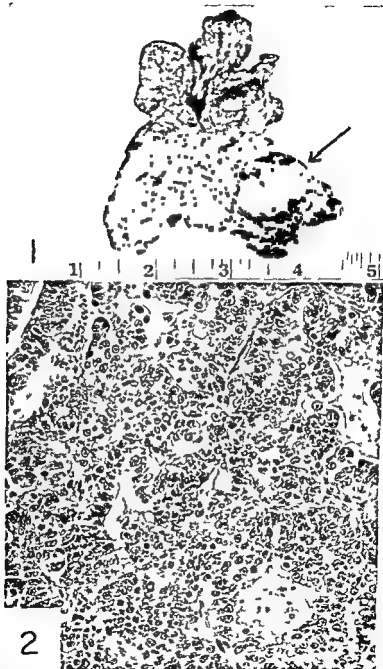
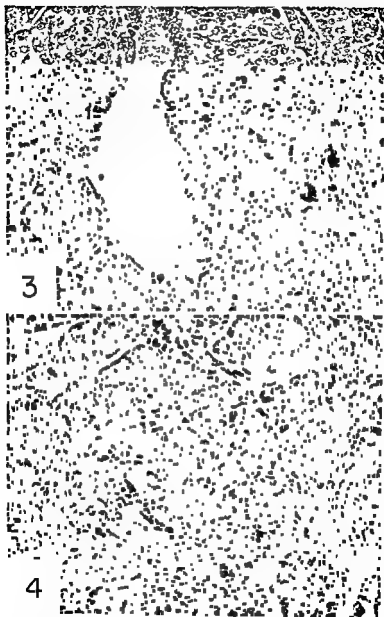


FIGURE 1. Photograph showing the typical gross appearance of a liver tumor from a choline-deficient rat. Reduced to three-fourths actual size.

FIGURE 2. Photomicrograph of a section of the liver tumor illustrated in FIGURE 1. In this area the tumor cells form ductlike structures. Hematoxylin and eosin stain. $\times 230$.



being recut. In the process of this study, two cases of pulmonary metastases from liver tumors have been found. In each case, an embolus was found in a blood vessel in the lung in addition to the metastatic nodule of tumor tissue. A photomicrograph of an embolus of tumor tissue (from liver tumor) in a blood vessel in the lung of a choline deficient rat (no. 14065) is shown in FIGURE 5. The pulmonary metastasis from this liver tumor is illustrated in FIGURE 6. A pulmonary blood vessel containing an embolus of tumor cells (rat no. 14067) is illustrated in FIGURE 7. The pulmonary metastasis from a liver tumor in this rat is shown in FIGURE 8.

Although only two cases of pulmonary metastases from liver tumors have been found to date in our choline deficient rats, this serves to prove that at least some of these tumors are malignant. The microscopic appearance of the two metastasizing tumors did not differ materially from many of those in which no metastases were found.

Histopathology of the Livers of Chronic Choline Deficient Chickens

Microscopic studies of slides prepared from the tissues of 105 chronic choline-deficient chickens and 33 control chickens fed the same diets supplemented with 0.3 to 0.6 per cent choline chloride have been made. The chickens remained on the choline deficient diet 28 to 133 weeks (average 78 weeks). The control chickens remained on the diet for 44 to 133 weeks (average of 80 weeks). Of the 105 chickens, 38 developed neoplasms of various kinds which were multiple in 12 chickens. No neoplasm was found in the 33 control chickens.

Fatty liver and cirrhosis. There was considerable variation in the gross appearance of the livers of the chronic choline deficient chickens. The livers varied in size, the majority of them being very large and fatty. An interesting feature of the fatty livers was that the entire liver was usually not involved. Scattered areas on each lobe frequently were of a normal, dark red color, the remainder of the liver having a light colored, fatty appearance. This was confirmed by microscopic studies of slides which showed some areas of normal liver tissue immediately adjacent to but sharply demarcated from, extremely fatty areas (FIGURE 9). The normal areas may represent regenerated liver tissue although they had the appearance of unchanged hepatic parenchyma. Gross nodular cirrhosis so frequently found in the livers of choline deficient rats was observed in only two cases, but frequently was observed microscopically (FIGURE 10).

Neoplasms of liver. Lesions resembling tumors were found in 23 cases. Eleven of these were classified as atypical regeneration nodules, 10 as cholangiocarcinoma and 2 as hemangioendothelioma. The gross appearance of one of the liver tumors is illustrated in FIGURE 11. The tumors varied in size from 0.8 to 3 cm. in diameter. The microscopic appearance of the liver tumors is illustrated in FIGURES 12, 13, and 14. There was a difference in the arrangement of the cells in different parts of the tumors varying from closely packed cells without any apparent order to well formed glandular and ductal structures. In some cases the neoplastic cells closely resembled liver cells, but in most cases they were anaplastic. FIGURE 12 illustrates one of the tumors in which

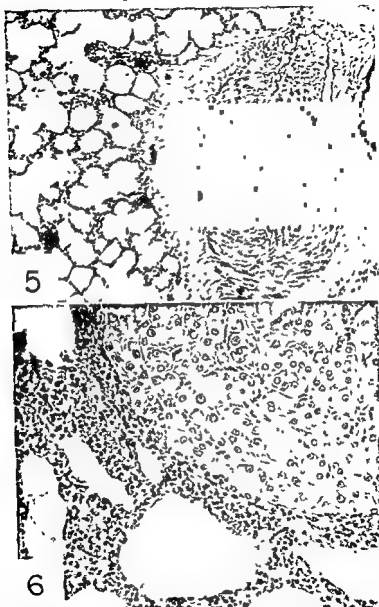


FIGURE 5 Photomicrograph of a blood vessel in the lung of choline-deficient rat no. 14065 containing an embolus of tumor cells from a hepatocellular carcinoma of the liver. Hematoxylin and eosin. $\times 150$.

FIGURE 6 Photomicrograph of a pulmonary metastasis from a liver tumor in choline-deficient rat no. 14065. Hematoxylin and eosin. $\times 310$.

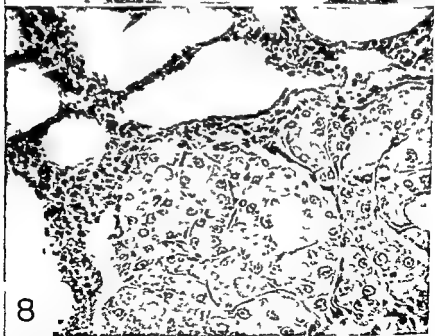
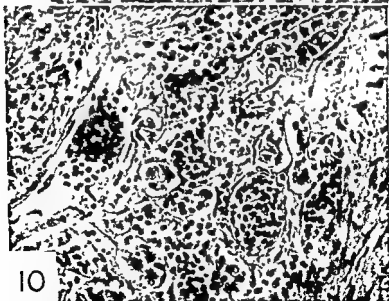


FIGURE 7. Photomicrograph of an embolus of tumor cells in a blood vessel in the lung of choline-deficient rat no. 14067. In situ liver tumor. Hematoxylin and eosin. $\times 275$.

FIGURE 8. Photomicrograph of a pulmonary metastasis from a liver tumor in choline-deficient rat no. 14067. Hematoxylin and eosin. $\times 10$.



9



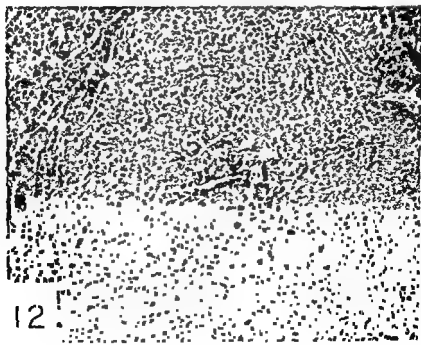
10

FIGURE 9. Photomicrograph of a section of liver from chicken 113 to illustrate extremely fatty conditions surrounding areas in which the parenchyma is normal. This may represent regenerated liver tissue. Hematoxylin and eosin. $\times 210$.

FIGURE 10. Photomicrograph of liver from chicken 211. This illustrates one of the cirrhotic areas which were found in the livers of chronic choline deficient chickens. Hematoxylin and eosin. $\times 430$.



11



12



the cells are so compactly arranged that cytoplasmic borders are not visible. The cytoplasm of the tumor cells does not stain as dark as the adjacent normal cells. The nuclei have irregular shapes and contain one or two nucleoli. In all the tumors, there were areas in which the cells were arranged in alveolar

Periodic acid stain revealed less glycogen than the normal like structures contained considerable amounts of pink staining material, probably mucus, thus being compatible with ductal origin of the tumor. Mucicarmine stains demonstrated only a small amount of mucus. Mucus was not demonstrable in atypical generation nodules or carcinomas with closely packed cells. In slides prepared by Foot's modification of Bielschowsky's reticulum stain, there was a heavy reticulum network throughout these tumors with a characteristic alveolar pattern. The reticulum fibers occasionally extended between individual cells in the epithelial island, but characteristically did not. No fibroglia were demonstrable in the sections of liver tumors stained with phosphotungstic acid hematoxylin.

Increase in Iron Positive Pigment in Liver and Kidney of Chronic Choline Deficient Chickens

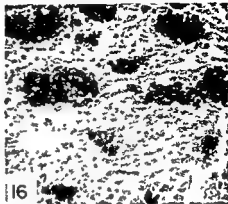
In studies of the microscopic appearance of tissues of chronic choline deficient chickens, an excessive deposition of pigment was observed in the livers and kidneys. Large accumulations of intracellular and extracellular pigment were observed in the livers, but in the kidneys the pigment was mostly intracellular. The intracellular hepatic pigment was occasionally seen in histiocytes but primarily in epithelial cells. In sections stained with hematoxylin and eosin, pigment was found in the form of yellowish brown granules varying slightly in size and depth of color and occasionally appearing as conglomerate masses.

Iron stains. The pigment gave a positive reaction for iron (Turnbull blue). A photograph of an entire section of liver illustrating the positive reaction is shown in FIGURE 15. It is of interest to note that the atypical generation nodule in the center of this section did not contain pigment, but there was a large amount distributed throughout the rest of the liver. A similar distribution was observed in the case of the livers that contained tumors. A slide of liver containing a large amount of intracellular and extracellular pigment is shown in FIGURE 16. The pigment in the epithelial cells is in the form of fine granules, but the extracellular deposits are large and small aggregates of granules. The general distribution of this pigment is illustrated in FIGURE 17. A photomicrograph illustrating the deposition of iron positive pigment in the convoluted tubules of the kidney is shown in FIGURE 18. A slide illustrating the difference in the amount of pigment in the convoluted tubules and collecting ducts of the kidney is shown in FIGURE 19.

The pigment gave the positive stain reactions for iron and was, therefore,



15



16



17



19



18

bility was further eliminated by the fact that tissues fixed by three different fixatives gave the same reaction

Discussion

The manifestations of choline deficiency in the chicken were somewhat different from those of the rat under the conditions of our experiments. In contrast

to the uniform accumulations of fat in the livers of rats in early stages the chicken livers were not uniformly fatty. In some cases entire lobes of a chicken liver contained very little fat even microscopically. This feature may account for the fact that nodular cirrhosis was found to involve the entire liver in only two cases and that in the majority of cases only scattered areas of the livers were cirrhotic.

A definite hemochromatosis (defined as hepatic cirrhosis with iron deposits in the liver and other organs) developed in approximately 25 per cent of the choline deficient chickens while more than normal quantities of iron positive pigment were found in the livers and kidneys of nearly all the birds. Slides of the liver and kidney from control chickens fed the same diet but with choline added were carefully checked for the presence of the pigment. There was a small amount present in some of the older chickens. The amount of pigment in the controls was extremely small in comparison to the large amount in the deficient chickens. In contrast to the excessive pigmentation of the livers and kidneys of chronic choline deficient chickens the livers and kidneys of choline deficient rats showed only moderate amounts of the pigments. The livers and kidneys of the choline deficient rats however did show a definite increase in iron positive pigments over the livers and kidneys of the control rats. Only minute traces of the pigment could be seen in livers and kidneys of the control rats.

Warren and Drake² have discussed the relationship between hemochromatosis and primary carcinoma of the liver in man and have published a pertinent bibliography. Gillman, Mandelstam, and Gillman³ have described the presence of large amounts of iron pigment in human cirrhotic livers in Africans whose diets appeared to be inadequate in quality.

It should be pointed out that the diets of the rats and chickens were free of vitamin B₁₂ and that the rat diets were also deficient in folic acid. It has been shown in this laboratory that vitamin B₁₂ has a definite choline sparing action.^{9, 10, 11} We have not found tumors in the livers of rats receiving choline deficient diets supplemented with vitamin B₁₂.

Summary

(1) Studies have been carried out on chronic choline deficient rats and chickens. The most prominent lesions have developed in the livers and kidneys of these animals.

(2) In the rat liver fatty infiltration has developed consistently and has been followed eventually in a large number of cases by cirrhosis and in some cases by tumor formation. Pulmonary metastases from liver tumors were found in two rats.

(3) In the chicken liver focal rather than generalized reactions were usually observed. Fatty changes and cirrhosis were common. Lesions considered to be hepatic malignancies developed in 12 cases with 11 others showing atypical regeneration nodules.

(4) Increased pigmentation with an iron positive pigment (hemosiderin) was observed in the livers and spleens of the choline-deficient rats and chickens but was much more severe in the chickens.

References

- 1 COPELAND D H & W D SALMON 1946 The occurrence of neoplasms in the liver, lungs and other tissues of rats as a result of prolonged choline deficiency *Am J Path* 22 109-79
- 2 ENGEL, R W D H COPELAND & W D SALMON 1947 Carcinogenic effects associated with diets deficient in choline and related nutrients *Ann N Y Acad Sci* 49 49-67
- 3 SCHAEFER A F, D H COPELAND W D SALMON & O M HALE 1950 The influence of choline deficiency on the development of the cirrhosis produced by diethylstilbestrol in the rat *J Natl Cancer Inst* 41 233-44
- 4 SCHAEFER A F, W D SALMON & D R STRENGTH 1949 Relation of vitamin B₁₂ to the development of the cirrhosis produced by diethylstilbestrol in the rat *Proc Soc Exp Biol Med* 71 202-204
- 5 SCHAEFER A F, W D SALMON & D R STRENGTH 1949 Interrelationship of vitamin B₁₂ and choline in the growth of the chick *Ibid* 71 202-204
- 6 GILLMAN J J, MANDELSTAM, & T GILLMAN 1945 A comparison of chemical and histological estimations of iron and copper content of livers of Africans in relation to the pathogenesis of siderosis and cirrhosis (hemosiderosis) *S African J Med Sci* 10 109-136
- 7 GILLMAN J J, MANDELSTAM, & T GILLMAN 1945 A comparison of chemical and histological estimations of iron and copper content of livers of Africans in relation to the pathogenesis of siderosis and cirrhosis (hemosiderosis) *S African J Med Sci* 10 109-136
- 8 GILLMAN J J, MANDELSTAM, & T GILLMAN 1945 A comparison of chemical and histological estimations of iron and copper content of livers of Africans in relation to the pathogenesis of siderosis and cirrhosis (hemosiderosis) *S African J Med Sci* 10 109-136
- 9 SCHAEFER A F, W D SALMON & D R STRENGTH 1949 Relation of vitamin B₁₂ to the development of the cirrhosis produced by diethylstilbestrol in the rat *Proc Soc Exp Biol Med* 71 202-204
- 10 SCHAEFER A F, W D SALMON & D R STRENGTH 1949 Interrelationship of vitamin B₁₂ and choline in the growth of the chick *Ibid* 71 202-204
- 11 SCHAEFER A F, W D SALMON & D R STRENGTH 1949 Interrelationship of vitamin B₁₂ and choline in the growth of the chick *Ibid* 71 202-204

HEPATOMAS PRODUCED IN MICE BY FEEDING BENTONITE IN THE DIET

By J. Walter Wilson

Department of Biology Brown University Providence, R. I.

It is the purpose of this paper to report the occurrence of hepatomas in mice fed a complete semisynthetic diet mixed with an equal amount of bentonite by weight. The details of the experiments and the evidence that has led to the tentative conclusion that the hepatomas are the result, in part, of an induced choline deficiency are to be reported in detail elsewhere.^{1, 2} Bentonite is a clay mineral which is composed of silicate and alumina. It is of the type known as smectonite and is characterized by its ability to absorb water and swell.

Mice can adapt themselves to eating a diet containing half its weight of such inert material as Ruffex or barium sulphate with only a slight decrease in growth rate (FIGURE 1). Where bentonite makes up 25 per cent of the diet growth is also nearly normal, but with 50 per cent bentonite in the diet growth is almost completely suppressed (FIGURE 2). The mice continue to live however, and grow very gradually for several months, but do not reach a normal size. Since the mice eat as much food on the 50/50 Bentonite diet as on the 50/50 Ruffex diet, failure to grow is not due to inanition alone.

Histological studies reveal that after the first three weeks there is more or less fat in the livers (PLATE 1, 1), apparently depending on whether or not the mouse is gaining weight. This is in keeping with the general observation that even on a diet free of lipotropic substances the liver may not contain fat if the animal is not growing. The fat appears first as fine droplets but soon many of the liver cells are distended with single large drops. Eventually after three or four months there is some fibrosis with progressive loss of architecture (PLATE 1, 2), although typical 'hob nail' cirrhosis has not been seen. There is also the accumulation of masses containing a brownish pigment, apparently ceroid (PLATE 1, 3).

Hepatomas have been found in all but one of twelve mice that in three experiments have been kept over 200 days on the diet. These are typical benign hepatomas of mice much like those produced by repeated CCl_4 treatment or occurring spontaneously in C_{57}H mice.

The fatty livers, fibrosis, ceroid, and hepatomas present a picture much like that we have previously reported in mice maintained for several months on a diet low in protein and deficient in choline.² The symptoms develop more gradually and the mice maintain a more thrifty appearance. On a choline-deficient diet, at sporadic intervals individual mice suddenly become anemic and die, so that only a few have been kept on it long enough to develop hepatomas. It seems possible that in these sudden deaths, toxic materials produced by the intestinal flora may be involved. If so, they may be removed by the bentonite which might account for the more thrifty condition of animals on the 50/50 bentonite diet.

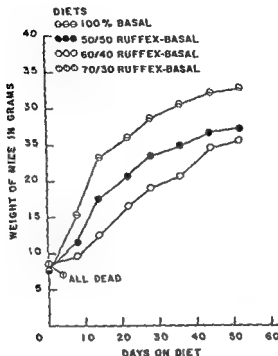


FIGURE 1

We have found that added choline will prevent the development of the fatty livers on the 50/50 bentonite diet, but that normal growth is only partially restored (FIGURE 2). When the amount of casein is doubled at the expense of corn starch, growth is also partially restored and the livers do not become fatty (FIGURE 3). This would be true of choline deficiency in both cases. When 1 per cent dl methionine is added to the 50/50 bentonite basal diet, however, the mice develop fatty livers and fail to grow (FIGURE 4). As a tentative explanation of this anomalous result, we suggest that the bentonite removes dibasic amino acids, and that the added methionine precipitates an incipient

that described for rats deficient in essential amino acids, which we have not as yet been able to cure.

It seems then that the deficiency is not a simple choline deficiency but may involve other essential basic materials removed from the intestinal contents by bentonite. It would be expected that some of the larger basic molecules would be taken up and held more firmly than inorganic cations, for with

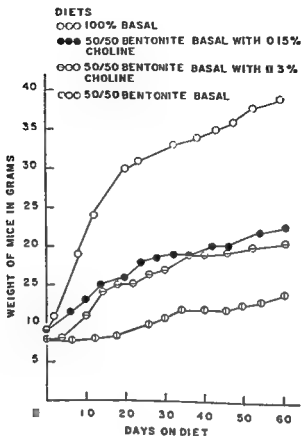


FIGURE 2

them, in addition to the Coulo and bases so held are only predict what would be tak the passage of the digestive bilities are under way

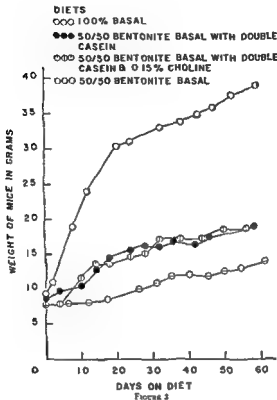
Except for the appearanc for the first four weeks. By size of cells a and region animal any mo the enla Similar l

rees Van der Waals f ulty eluted. It w uch complicated riments designed

are no strikin ere is a pron bular area homogen icious in cuoles re n gro i

into play ifficult to s occur in us possi

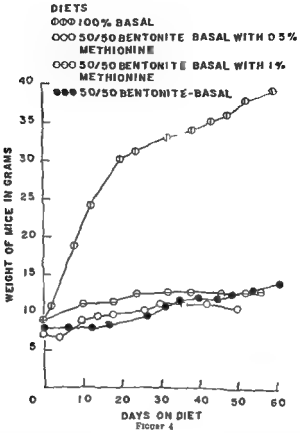
es



mice when they have been fed a diet containing a carcinogenic azo dye for a few weeks⁶

enlarged liver cells are placed on a normal diet, rapid growth resumes and a spectacular wave of mitosis occurs in the liver which involves the large cells as well as the small ones (PLATE 2, 3). The large nuclei are obviously polyploid for they have very wide angled spindles and very large numbers of chromosomes. The metaphase illustrated in PLATE 2, 3 has a few chromosomes that have failed to reach the equatorial plate. From their size the numbers on the plate must be great. T_h — — — — —

Many of the figures are anaphase and telephase (PL.



this sort in other cases where mice also when the liver, regenerating. Whether they result in viable cells and thereby play a role in carcinogenesis, speculation, and is worthy of further observation of Hauschka and Levy's work on ascites tumors of

the mouse liver⁶ after hepatectomy, chromosome analysis suggests⁷ is particularly interesting in view of abn

	P	
1 Wilson's disease	Nutritional deficiency	reduced
2 Wilson's disease	Inst 14 57	contain
3 Wilson's disease	Hepatomas in	low
4 Hepatic carcinoma	malnutrition	
	exchange	
	in adenosine	

5. SHIMIZU, Y. 1951. The effect of bentonite on the growth of the mouse. *J. Nat. Hist. Mus. Tokyo* 46: 1-10.
6. WILSON, J. H. 1948. The effect of bentonite on the growth of the mouse. *Am. J. Hyg.* 58: 1-10.
7. BRUNS, A. M. & L. REITZ. 1951. Effects of external and internal radiation on cell division. *Ann. N. Y. Acad. Sci.* 61: 1497.
8. HAUSCHKA, T. & A. LEVAN. 1952. Chromosome numbers of three mouse ascites tumors. *Hereditas* 40: 251.

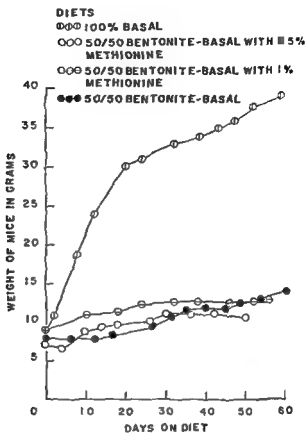


FIGURE 4

this sort in other cases where mitosis occurs in the mouse liver.⁶ They occur also when the liver, regenerating after partial hepatectomy, is irradiated. Whether they result in viable cells with abnormal chromosome complements and thereby play a role in carcinogenesis as Brues suggests,⁷ is an interesting speculation, and is worthy of further investigation particularly in light of the observation of Hauschka and Levan⁸ on the occurrence of abnormal chromosome numbers in ascites tumors of mice.

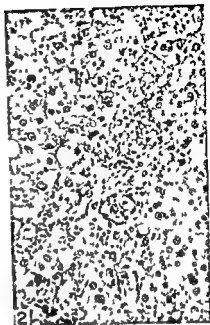
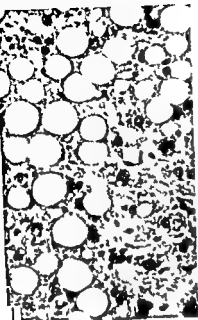
References

- 1 WILSON, J. W. 1953 Nutritional deficiency in the mouse produced by bentonite feeding. *J. Nat. Cancer Inst.* 51.
- 2 WILSON, J. W. 1953 Hepatomas in mice on a bentonite containing diet. *J. Nat. Cancer Inst.* 14: 63.
- 3 WILSON, J. W. 1953 The effect of bentonite on the growth of mice on a protein and deficient diet. *J. Nat. Cancer Inst.* 14: 63.
- 4 HILG, J. 1953 The effect of bentonite on the growth of mice on a protein and deficient diet. *J. Nat. Cancer Inst.* 14: 63.

- 5 SHELTON F 1949 *A cytological and cytochemical study of the liver of the mouse feeding M Methyl P Dimethylaminazobenzene* Thesis Brown University
- 6 WILSON J W & E H LEDUC 1950 Abnormal mitosis in the mouse liver *Am J Anat* 51
- 7 BRUES A M & L REITZ 1951 Effects of external and internal radiation on cell division *Ann N Y Acad Sci* 51 1497
- 8 HAUSCHKA T & A LEVAN 1952 Chromosome numbers of three mouse ascites tumors *Hereditas* 38 251

PLATE 1

1 Fatty liver of mouse on 50:50 bentonite basal diet with 1 per cent methionine for 49 days. Ocular



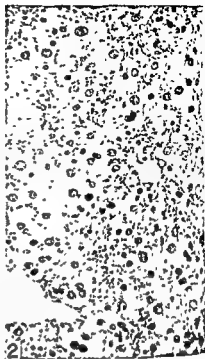
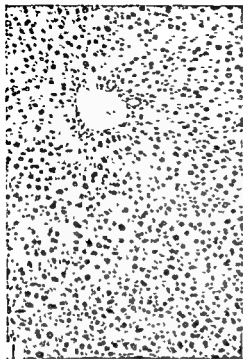


PLATE 2

Cholesterol - mg %
Alkaline Phosphatase Units

No 2155

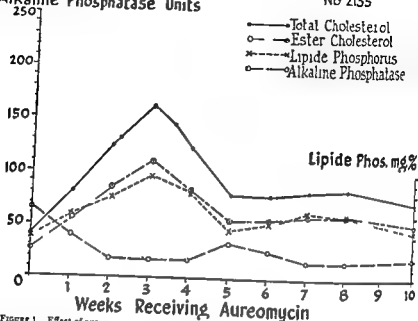


FIGURE 1. Effect of aureomycin upon the serum lipids and alkaline phosphatase activity of pancreatic duct ligated dog no. 2155.

regression took place and the concentrations of serum lipids leveled off at approximately 50 per cent of the peak values. These were still well above the control concentrations. The serum alkaline phosphatase activity rose somewhat during this period but did not approach the initial value.

Dog No. 2661 The pancreatic ducts of this dog were ligated 44 weeks prior to the antibiotic experiment. Like the preceding animal, this dog responded to aureomycin as follows:

serum

in four weeks but were not as marked as in the previous animal, corresponding more closely to the plateau values. These alterations, however, were sustained for an additional 10 weeks at which time aureomycin was withdrawn from the diet. There was no marked change in the serum cholesterol and alkaline phosphatase.

Dog No. 1921

previous to this:

showed the customary low blood lipids and high serum alkaline phosphatase activity (FIGURE 3). Three weeks after the administration of aureomycin the serum cholesterol and alkaline phosphatase

returned to the control and peak levels. At this time the animal was given daily 6 mcg of vitamin B₁₂ subcutaneously and 5 mgm. of

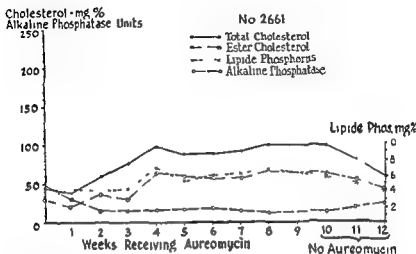


FIGURE 3. Effect of aureomycin *per os* upon the serum lipids and alkaline phosphatase activity of pancreatic duct ligated dog no. 2661.

folic acid orally in addition to the aureomycin. The serum cholesterol and lipid phosphorus promptly rose to peak levels and were maintained at this point for three weeks. Alkaline phosphatase activity also fell to its lowest level. Upon withdrawal of the aureomycin from the diet while continuing the above vitamin administration, the serum lipids fell and the alkaline phosphatase activity rose. These results indicate that the lipotropic action of aureomycin exceeds that of the vitamin B₁₂ and folic acid. It should also be pointed out that, one year previously, no effect on serum lipids or alkaline phosphatase activity was obtained in dog 1921 or in one other animal by the oral administration of 25 mcg of vitamin B₁₂ plus 5 mgm of folic acid, nor by the subcutaneous administration of 100 mcg of vitamin B₁₂ alone, or 6 mcg of vitamin B₁₂ plus 4.5 mgm of folic acid. No antibiotics were given at that time.

In searching for the mechanism by which aureomycin was exerting its lipotropic effect, consideration was given to the possibility that, through its antibacterial action, less methyl acceptors may be formed. Although little is

known about the mechanism of action of ergothioneine. The possibility of other methyl acceptors being involved in this condition cannot be eliminated.

In view of the reports by Huerga and Popper^{28, 29} that the major portion of a test meal of choline is converted to trimethylamine and its oxide by intestinal bacteria and that this conversion can be temporarily inhibited by pretreatment

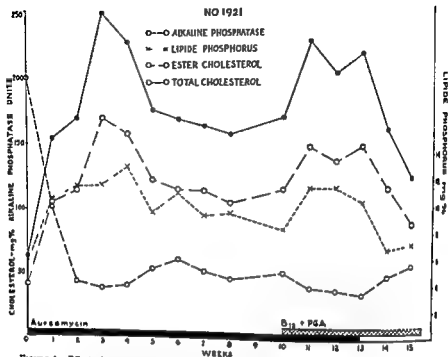


FIGURE 3. Effect of aureomycin alone and in conjunction with vitamin B₁₂ plus folic acid upon the serum lipids and alkaline phosphatase activity of pancreatic duct ligated dog no. 1921.

TABLE 1
THE EFFECT OF AUREOMYCIN INGESTION UPON THE EXCRETION OF ERGOTHIONEINE BY DOGS WITH LIGATED PANCREATIC DUCTS

Weeks receiving aureomycin*	Weekly excretion of ergothioneine (mg)		
	Dog 2155	Dog 2661	Dog 1921
none	862	810	725
1	1254	598	978
2	900	1078	888
3	1652	1444	655
5	1011	1373	
6			922

* 1.0 g. daily for the first 3 weeks and 0.75 g. daily for the remainder.

with antibiotics, some measurements* of total trimethylamine excretion were made in some of our treated animals. No consistent depression of cholesterol destruction was found during prolonged aureomycin therapy. If less cholesterol was being converted to trimethylamine or its oxide, the action was temporary and of insufficient magnitude to explain the lipotropic effect of the antibiotic.

Discussion

These limited data indicate that aureomycin does have a lipotropic action when given *per os* to dogs with ligated pancreatic ducts, as shown by a marked

* We are indebted to Dr. J. de la Huerza of Hektoen Institute, Chicago, Illinois, for these analyses.

rise in serum cholesterol and phospholipid levels and a dramatic fall in serum alkaline phosphatase activity. Conclusive demonstration of a lipotropic effect, however, depends upon liver biopsy studies. Such experiments are under way.

The inability of aureomycin to maintain a normal level of serum cholesterol and phospholipids as do lipotropic substances such as choline or methionine, may be explained by partial bacterial adaptation to the antibiotic. In view, however, of recent reports^{20, 21} that the administration of aureomycin or terramycin may cause a fine, fatty infiltration of the liver this latter possibility has to be taken into consideration. Nevertheless, it appears from our limited data⁸ that fatty infiltration of the liver induced in the dog by the antibiotic *per se* is minimal if present at all but more animals are needed to settle the question. This is in agreement with the finding of Lepper *et al*²² that a very large oral dose of antibiotic is required to produce fatty liver changes in mice.

Gyorgy and his associates were the first to demonstrate that aureomycin and other antibiotics exert a lipotropic action when fed to rats receiving a low protein low choline, high fat diet² and that the same antibiotics delay the onset of liver necrosis when rats are fed a necrogenic diet.^{19, 21} The recent

in rats suffering from a choline deficiency or in dogs with a deficiency of pancreatic exocrine secretions is associated with its antimicrobial action. This

tion of choline and methionine by the bacteria themselves.

Schaefer *et al*²⁴ and Burns and McKibben²⁵ have reported a lipotropic effect

aureomycin, however, at a time when the lipotropic effect of the latter had

under the same conditions. This suggests that the degree of choline deficiency or the bacterial flora of the intestine may be factors influencing the lipotropic effect of vitamin B₁₂ and folic acid.

Summary

(1) The oral administration of aureomycin to dogs with ligated pancreatic ducts has a lipotropic effect as manifested by a marked rise in the existing low concentrations of serum cholesterol and lipid phosphorus to values approaching normal. The elevated serum alkaline phosphatase activity falls sharply

* A normal dog was given by mouth 1.0 gm. aureomycin daily for 3 weeks and 0.75 gm. daily for an additional 2 weeks before it was sacrificed. Total liver lipids were at the upper limit of normal, namely 4.9 per cent.

(2) These lipid and enzyme changes reach their maximum in three weeks and then recede and level off at some intermediate value.

References

- 1 ALLAN, F N D J BOWIE, J J R MACLEOD, & W L ROBINSON 1924 The effect of the components of leucine on the metabolism of the liver and autoclaved pancreas maintained with insulin
- 2 KAPLAN A & I L CHAIKOFF 1937 The effect of choline on the lipid metabolism of the liver in the completely depancreatized dog maintained with insulin Biol Chem 120 647
- 3 CHAIKOFF, I L C ENTENMAN & M L MONTGOMERY 1945 The mechanism of the effect of choline on the lipid metabolism of the liver in the completely depancreatized dog J Biol Chem 160 199
- 4 HAANES M L & P GYORGY 1951 In vitro action of a new lipotropic fraction of pancreas Am J Physiol 166 441
- 5 RHOADS J E O LIBORO S FOX P GYORGY & T E MACIELLA 1951 action of a new lipotropic fraction of pancreas Am J Physiol 166 436
- 6 FEINBERG H I L CHAIKOFF & C ENTENMAN 1952 Antifatty liver: papain and ficin in insulin treated depancreatized dogs Proc Soc Exptl Biol Med 80 161
- 7 RALLI E P & S H REBEN 1942 The effect of meat and meat fractions on liver of the depancreatized and pancreatic duct ligated dog Am J Physiol 136 101
- 8 GYORGY P J STOKES JR W H SMITH & H GOLDBLATT 1950 Studies on the effect of aureomycin in hepatic disease II The effect of aureomycin on experimental dietary hepatic necrosis Am J Med Sci 220 6
- 9 GYORGY P J STOKES JR H GOLDBLATT & H POPPER 1951 Antimicrobial activity of aureomycin in the prevention of dietary hepatic injury (necrosis cirrhosis) in rats J Exp Med 153 513
- 10 GYORGY P 1952 The effect of aureomycin on the liver of the depancreatized dog J Biol Chem 197 101
- 11 SPERRY W M & M M CHOLESTEROL DETERMINATION BY THE FOLIO-CROCKFORD METHOD
- 12 SCHOENHEIMER R & J M WELSH 1934 The determination of cholesterol in biological materials by the Lieberman-Burchard reaction J Biol Chem 107 155
- 13 ZILVERSMIT D H & J M WELSH 1934 The determination of cholesterol in biological materials by the Lieberman-Burchard reaction J Biol Chem 107 155
- 14 KAPLAN A & A NARAHARA 1953 The determination of serum alkaline phosphatase activity J Lab Clin Med 41 819

SURVEY OF THE WORLD SITUATION ON KWASHIORKOR

By J F Brock

Department of Medicine, University of Cape Town Cape Town, Union of South Africa

A world survey is of value in this scientific monograph because the study of the ecology and epidemiology of kwashiorkor* may provide useful clues to the etiology and pathogenesis of cirrhosis and primary carcinoma of the human liver

The term "kwashiorkor" now has, for better or for worse, the sanction of the Joint F A O / W H O Expert Committee on Nutrition, which has quoted and presumably endorsed the view expressed by Brock and Autret that a wide and general appraisal of its nature and relationships suggests that it is the most serious and widespread nutritional disorder known to medical and nutritional science" In the African continent, the term has been accepted almost unanimously as replacing "malignant malnutrition," "infantile pella gra," "diboba," "mbuaki," and other local names

No attempt will be made in this communication to trace the history and details of this syndrome and its bibliography except in so far as they are relevant to the title of this monograph Brock and Autret⁶ have done this and have made acknowledgment to the pioneers in this field in Africa They defined kwashiorkor as seen in the African continent in the following terms "It is recognized that the syndrome cannot at present be accurately defined and that it merges into other nutritional syndromes, such as marasmus and Mehlmanrschaden, but it is felt that its prevalence and importance justify a tentative definition as follows

"A nutritional syndrome (or syndromes) found among indigenous Africans in which characteristically there occurs

- "(a) retarded growth in the late breast feeding, weaning, and post weaning ages, with
- "(b) alterations in skin and hair pigmentation,
- "(c) oedema
- "(d) fatty infiltration, cellular necrosis, or fibrosis of the liver,
- "(e) a heavy mortality in the absence of proper dietary treatment, and
- "(f) the frequent association of a variety of dermatoses

"Some combination of the above features appears to be fundamental other clinical features are so frequent as to be dominant if not fundamental"

The African aspects of kwashiorkor were brought up to date by the Second Conference on Nutrition called by the Commission for Technical Cooperation in Africa South of the Sahara (C C T A) in November 1952 at Fajara Gambia West Africa⁷ The Joint F A O / W H O Expert Committee on Nutrition¹⁸ held its third session at Fajara immediately after the C C T A Conference The members of the Committee had the advantage of hearing

graph is
called
in V oc
tion of

the full discussion at the C C T A Conference and subsequently considered kwashiorkor in relation to the problems of nutrition in mother and child throughout the world

In the light of these two meetings, the definition and description given by Brock and Autret can be modified or expanded in certain respects. The urative effect of reconstituted skim milk powder has been amply confirmed and this might be listed as a fundamental and often critical diagnostic feature as predicted by Brock and Autret, vegetable protein has proved to be capable of substitution to a limited extent for milk protein.¹⁰ We can conclude, therefore, that the deficient factor or factors are among the constituents of dried skimmed milk. Whether the deficiency pattern is (1) a simple disproportion between total protein and calories, or (2) an amino-acid imbalance, or (3) a deficiency of accessory food factors yet to be identified, remains an open question. The Joint F A O / W H O Expert Committee on Nutrition has very properly left this matter open in its tentative definition of "protein malnutrition".

There is no further confirmation of the claim by Auffret and Tanguy¹ that the milk of African mothers is deficient in methionine. Bigwood² has pressed the importance of cystine deficiency. Trowell, Davies, and Dean³ have brought their description of kwashiorkor in Uganda up to date. Davies⁴ has given an account of the pathology of the syndrome as seen in Uganda. He believes that certain features are pathognomonic, especially the pancreatic lesion and the 'stellate' fibrosis of the liver. He holds that atrophy of the acinar cells of the pancreas with marked decline in the extrinsic secretions of the gland is a fundamental feature of the syndrome and perhaps the essential and earliest lesion. If Davies' observations and interpretations are correct, and if they apply to kwashiorkor as seen in other regions then the pathogenesis of the syndrome can be stated in some such terms as the following. Under the influence of protein malnutrition which is acutely precipitated by weaning, the pancreas and other organs of exocrine secretion are first affected. This leads to decline in enzymatic and other efficiency of the gastrointestinal tract and creates a vicious circle of conditioned and dietary protein deficiency, which, in turn, affects the liver and every tissue in the body.

Turning from Africa to other parts of the world the incidence of kwashiorkor was reviewed by Meiklejohn and Passmore⁵ and has recently been brought up to date.¹⁰ A survey of kwashiorkor in Central America has been made by Autret and Behar and will be reported. There is a general impression that kwashiorkor is a syndrome apparently basically the same as "culebrilla" syndrome pluricarenal, "syndrome hipoproteimico" and related syndromes of Central and South America and the "fatty liver disease" of Jamaica. Apart from the pigmentary disturbances of the skin which are usually present it is probably basically the same as the 'Mehlnährschaden' of Germany and Austria in the early 1900's, and the 'dystrophie de farineux' and 'dystrofia de farinacei' still recognized in the unpigmented or lightly pigmented children of southern Europe. The report of the third session of the Joint Committee (November 1952) contains a comprehensive list of titles which the Committee believes to signify syndromes of the same nature as

kwashiorkor and covered by the general term "protein malnutrition" as defined in the report "a state of ill health occurring where diets are habitually poor in good quality protein, while they are more nearly adequate in calories. It includes deficiency of protein foods, imbalance of amino acids, and deficiency of accessory food factors associated with protein metabolism. It is most easily recognized when calorie intakes have been relatively high from the use of starchy foods."

Because no one name commands general acceptance to cover the "protein malnutrition" syndromes of the American tropical belt and because the Joint F A O / W H O Expert Committee on Nutrition has been inclined to include them under the heading "kwashiorkor," I have compromised in this communication by grouping them for convenience under the term "American kwashiorkor." I could not use the plain term "kwashiorkor" because on account of the apparent difference in sequelae, I have asked the question whether kwashiorkor and "American kwashiorkor" are the same disease? In spite of difficulties which will be discussed, I believe the answer to be in the affirmative.

A brief summary will be given of present knowledge in regard to the liver lesion or lesions associated with kwashiorkor. This falls under two headings: (1) the pathology of the liver in kwashiorkor in childhood, and (2) the liver lesions of adults in the areas of prevalence of kwashiorkor and protein malnutrition and the possible relationships between these adult lesions and those of the children. Brock and Autret reported that the liver pathology of African kwashiorkor consisted of some combination of fatty infiltration, necrosis, and fibrosis. These three features showed various combinations, and there is much experimental evidence that all three may occur separately and independently as manifestations of dietary deficiency. The experimental evidence for the separate origins of fatty infiltration and necrosis are reviewed elsewhere in this monograph.[†] There can be no doubt that the processes described under these two terms are quite distinct, but it should be emphasized that even the "dissolution of fatty cysts" described by Hartroft in his lipohepatitis is a form of death of cells. It was in this wide sense that the liver lesion of kwashiorkor was described by Brock and Autret as "fatty infiltration, cellular necrosis, or fibrosis." It was intended to leave the pathogenesis an open question. The available evidence certainly suggests close analogies between the liver lesion of kwashiorkor and experimental fatty liver cirrhosis but it would be premature to press this comparison too far. This subject is discussed further in the section on definitions (Appendix, p. 709). Workers on kwashiorkor in Africa have inclined strongly to the concept of the independence of fatty infiltration and of fibrosis. Both Gillman¹² and Davies¹ have recorded their views on this subject. There seems to be no reason why the fatty infiltration of kwashiorkor should not be permanently reversible on provision of a proper diet, and the problem of continuing fibrosis is discussed in a later section of this communication.

Brock and Autret in their report stated that "it can be accepted as reasonably probable, from their world distribution, that cirrhosis and primary car-

[†] See page 623

cinoma of the liver on the one hand, and kwashiorkor on the other hand, are aetiologicaly related. The evidence strongly suggests that both are due to dietary deficiency, particularly deficiency of protein." The evidence for this statement is worth critical examination, since it is based on some tentative assumptions.

The areas of the world in which kwashiorkor is prevalent are very similar to but not identical with the areas in which cirrhosis and primary carcinoma of the adult human liver have a high incidence. The areas of correspondence are readily explainable on the attractive hypothesis that the gross hepatic damage of infantile kwashiorkor is the starting point of a fibrosis which proceeds insidiously throughout adolescence and presents itself clinically in the third and fourth decades of life as a "nutritional cirrhosis" which is often complicated by primary carcinoma of the liver. This hypothesis is at present only provisional. Careful examination of maps of the world areas of noncorrespondence between infantile kwashiorkor on the one hand and 'nutritional cirrhosis' and primary carcinoma of the liver in adults on the other may yield important clues as to the reliability of the assumption.

What is needed at present is a series of accurate maps of the world reflecting the areas of incidence of (1) kwashiorkor or related protein malnutrition syndromes, (2) prevalence of primary carcinoma of the liver, and (3) prevalence of cirrhosis of the liver. The first map has been constructed from the literature^{2, 3} but will need to be filled in as reports accumulate (FIGURE 1). A version of the second map is contained in Berman⁴ (FIGURE 2).

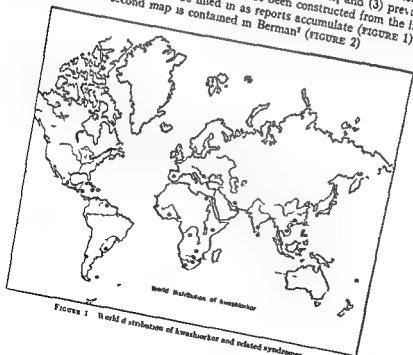


FIGURE 1 World distribution of kwashiorkor and related syndromes

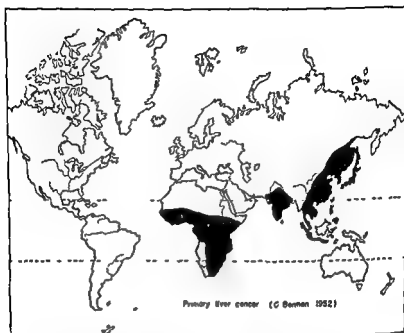


FIGURE 2 World prevalence of primary carcinoma of the liver

Without detracting from the value of Berman's work, this map can be criticized both artistically and scientifically in its "black and white" character. The figures given in FIGURE 3, prepared by myself, show that the difference in incidence of primary liver carcinoma could be better represented by a gradual merging from black through grey to white as we pass from the tropics through the pigmented residents of temperate climates to the European races. Berman's map can also be criticized on the score that he produces no evidence that the prevalence of primary carcinoma admitted to exist in South China continues up to North China as represented on his map.

If these two maps are compared, there is considerable correspondence between the latter and the world map of kwashiorkor as far as Africa, India, China, and the Far East within the tropical belt are concerned but a notable discrepancy with respect to the tropical belt of the American continents. Does this blank in the tropical portion of the map of incidence of primary liver cancer reflect actual conditions, or is it due to failure to record and report? Warui¹¹ said, "Near and Far Eastern races have a greater incidence of liver cell carcinoma than those of Europe or the Americas." Berman's summary confirms this, and apparently there is no contrary evidence.

I have sought unsuccessfully in the literature for information on this point and have questioned and corresponded with those who might know. The

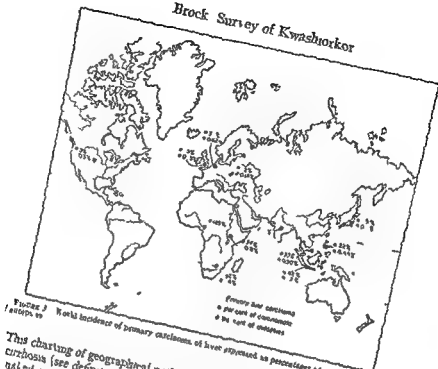


FIGURE 2
FIGURE 2
World incidence of primary carcinoma of liver expressed as percentages of all carcinoma and

This charting of geographical pathology is complicated by different concepts of cirrhosis (see definitions) but in general the figures represent incidence rates of less naked eye incidence. The open circles represent incidence rates of less than 1.5 per cent in general autopsies and the black dots higher rates. It will be seen that the difference between the tropical and nontropical areas is considerable but not as gross as would be expected from the map of primary liver cancer. For interest in relation to later discussion I have also charted the areas of prevalence of siderotic cirrhosis and of liver damage resulting from hepatotoxic alkaloids contaminating food.

The only data given for the American tropical belt are those derived from reports on the yellow fever viscerotome service a method whereby post mortem liver samples were obtained without doing an autopsy. The figure of 2 per cent for Colombia represents 404 cases with signs of cirrhosis out of 22 000 specimens. That of 0.5 per cent for central and northern Brazil represents 55 livers with advanced cirrhosis out of 29 592 specimens. It must be remembered that these were all microscopic specimens. If the data are based on the usual criteria the incidence of cirrhosis is low in these two large countries of the central American belt. I have been able to find no publications on the cirrhosis rate for this area based on the usual autopsy incidence rate.

If microscopic criteria are taken for the diagnosis of cirrhosis then the incidence becomes very much higher in some parts of the world. The Gillmans

M827
3724



FIGURE 4. Liver cirrhosis expressed as a percentage of autopsies in areas of high and low incidence. Areas of liver damage associated with siderotic or iron rich cirrhosis and with toxic cirrhosis are shown.

for example, claim that among 261 adult African (Bantu) subjects dying "sudden and rapid accidental death" only 12.6 per cent of livers were histologically normal. Such a figure cannot be compared with figures from other parts of the world, since histological examination of livers from subjects dying of other causes is not available.

than his macroscopic rate of 2.5 per cent.

On the other hand, Bauer and Kerr, although they record only 55 cases of "advanced cirrhosis" in 29,593 specimens, nevertheless record that, in 1594 of those specimens in which lesions of *S. mansoni* were found, "periportal reaction" is always present and ranges from a slight hepatitis through various degrees of fibrosis with chronic inflammation to severe cirrhosis. And in 2298 further specimens showing malarial pigment, there were 23 per cent with "periportal reaction." Admitting that periportal reaction is not synonymous with cirrhosis, it seems that the figure of 55 cases of "advanced cirrhosis" in the whole series represents a very conservative estimate of the actual incidence of cirrhosis in Brazil.

The gap covering the American tropical belt in the world map of cirrhosis of the liver constructed from published information is very obvious, and again the question arises, as in the case of the map of primary carcinoma, as to whether this gap is real or only apparent? The opinion of some of those who have had experience is that it is only apparent. Until it is filled in, it cannot finally be

concluded that "American kwashiorkor" is the same disease as kwashiorkor in Africa, for it appears, at present, not to have the same sequelae.

Brock. Survey of Kwashiorkor

70.

Interpretations

If there were any consequential relationship between kwashiorkor and primary carcinoma of the liver, that sequence would presumably be through the insidious development of cirrhosis of the liver as a sequel to kwashiorkor, because (1) the correspondence between primary carcinoma and cirrhosis of the liver is so close and (2) experimental evidence suggests that protein malnutrition in animals can result in progressive and permanent cirrhosis.

Two questions must be asked at this point (1) What is the evidence that kwashiorkor in African can produce permanent cirrhosis of the liver? (2) What is the evidence that "American kwashiorkor" can produce cirrhosis of the liver? It will be seen in the later discussion that a final answer cannot be given to either of these two questions.

The next question to be asked is: What other factors besides protein malnutrition may enter into the production of cirrhosis of the liver either as causal or contributory factors? In the later discussion, this aspect of the problem will be treated under the following headings: (1) viruses, (2) tropical parasites, (3) alcohol, (4) hepatotoxic alkaloids and other food contaminants, (5) high dietary iron intakes, and (6) genetic factors.

The question will then be asked whether any of these factors can explain the gap in the prevalence of carcinoma of the liver in the Central American belt where "American kwashiorkor" is prevalent and where protein malnutrition is believed to be widespread.

Finally the question will be asked whether kwashiorkor as seen in the area of world prevalence of primary carcinoma of the liver (i.e., in Africa, India, China and the Far East) can be regarded as essentially the same as "American kwashiorkor".

Does African kwashiorkor produce cirrhosis? There has been a fairly general assumption that African kwashiorkor does produce cirrhosis largely because of the assumed geographical correspondence between kwashiorkor on the one hand and of cirrhosis and primary carcinoma of the liver on the other. It must be emphasized, however, that this is only an assumption. The only real evidence on the subject that has been put on record is the opinion of Davies.⁸ In discussing the pathology of the liver in kwashiorkor he states "The localization of the fat is in the periphery of the liver lobule, often sparing the cells of the lamina limitans. As the disease progresses, the fat appears nearer and nearer to the central vein until the whole lobule is full of fat. With the increase in fat there is an infiltration of the portal tracts by round cells, and fine fibrosis appears around the periphery of the lobule, spreading out in a stellate fashion from the portal tracts. The fibrosis results from a thickening of the reticulum around the peripheral cells. It spreads throughout the liver until in severe cases a state resembling a Lacunae's cirrhosis is seen. If the child survives either with or without treatment, the fat tends to disappear slowly, first from the centrilobular region, and lastly from the periphery, leaving behind a fine stellate fibrosis and round cell infiltration in the portal tracts. Treat-

ment with protein leads to rapid fat reduction and a flooding of the sinus with lymphocytes, but does not affect the fibrosis and cell accumulation. These may persist for many years, if not for life, and in an African appear to be a "hallmark" of kwashiorkor. Why they should be so persistent, when it is well known that in other conditions quite well marked fibrosis of the liver can disappear, is a mystery, but it suggests that there is continuing liver damage of some sort. If this view is correct we must ask what the nature of the "continuing liver damage of some sort" referred to at the end of Davison's quotation is. Is it continuing protein malnutrition, or do other cirrhotogenic factors which will be discussed below play a part? No other definite evidence for the progressive evolution of cirrhosis of the liver out of African kwashiorkor has been recorded in the literature. The Gillmans¹³ say, "We have very little information about the reactions of the livers of Africans between the ages of six and eighteen years, because during this age period, for some still undetermined reason, temporary but acute episodes of nutritional disease are rare. Biopsy in one seven year old child revealed that the increase in hepatic iron may commence at this age. We know most certainly that the hepatic iron content increases rapidly after the age of eighteen years. As we have indicated, primary hepatocellular carcinoma may and frequently does supervene between the ages of sixteen and twenty years and may occur at an even younger age. In other parts of the world, progressive cirrhosis of the liver does not appear to be an inevitable sequel of syndromes resembling kwashiorkor. No evidence has yet been published that the Mehlinahrs Schaden and starch dystrophy of Europe progress to cirrhosis of the liver. In the following discussion, it will be seen that "American kwashiorkor" has not been shown to be associated geographically with a high incidence of adult liver cirrhosis. My colleagues, Doctor P. Suckling and Professor J. G. Thomson in Cape Town, have collaborated with me in searching in biopsy material for continuing evidence of cirrhosis of the liver in children recovered from kwashiorkor among the colored (non Bantu) population of Cape Town (Brock, 1949). So far,

In the

cirrhosis of the liver after it has been cured and provided that the subsequent diet is satisfactory and cirrhotogenic agents are not prevalent. On the other hand, there is very convincing evidence that, in certain parts of Africa, at least, fibrosis may persist for many years, if not for life, after clinical recovery from kwashiorkor in the presence of "continuing liver damage of some sort" and "increase in hepatic iron."¹³

Does American kwashiorkor produce cirrhosis? There seems at present to be very little, if any, evidence that American kwashiorkor produces cirrhosis. In fact, the absence in the literature of evidence for a high prevalence of primary carcinoma of the liver and of cirrhosis of the liver in the American tropical belt may be regarded as indirect evidence against such a supposition. This matter must, however, be left open until further evidence accumulates. Further accumulation of evidence should confirm that these two disorders

not prevalent, we must conclude either that "American kwashiorkor" is not the same as African kwashiorkor or that the "continuing liver damage of some sort" postulated by Davies in Africa is not prevalent in the American tropical belt.

What other factors besides protein malnutrition enter into the production of cirrhosis of the liver? One of the few things that are certain about cirrhosis of the liver is that it may result from several, if not many, causes. In all probability, the causation is frequently multiple. I will not examine the evidence on experimental nutritional liver damage in animals since it has been so thoroughly discussed in this monograph. Turning to the human being, I do not think anybody will dispute the role of malnutrition in the production of human liver damage. What is more relevant to our present discussion is to ask (1) Can any form of human malnutrition lead to a fibrosis which is permanent or progressive when the diet is restored to normality and when other cirrhotogenic agents are absent? (2) Can the effects of severe human malnutrition, as in kwashiorkor, be continued throughout the rest of life by milder degrees of malnutrition in the absence of other cirrhotogenic agents? (3) What other cirrhotogenic agents need be seriously considered in the causation of the cirrhosis which is so prevalent in the areas of high prevalence of primary carcinoma of the liver? The only reply that can be made to the first question is that there is, at present, no convincing evidence in the human being, and there is unlikely to be in the future. The answer must be sought by analogy from animal experiment. To the second question the reply must be made that both animal experiment and the study of the epidemiology of kwashiorkor suggest strongly that continuing protein malnutrition may be an important factor in determining the progressive evolution of adult cirrhosis out of kwashiorkor. If this assumption is true, why does it not also hold for the American tropical belt? In the light of this noncorrespondence, we must assume either that protein malnutrition is not continued into adolescence in the American tropical belt to the same extent or in the same form as in Africa, or else that cirrhotogenic agents other than malnutrition operate to produce adolescent cirrhosis in Africa but not in central America.

The possible or probable cirrhotogenic agents will now be discussed in order (1) *Viruses*. There can be no doubt that viruses may act as cirrhotogenic agents. It may well be argued that they act only as cirrhotogenic agents when other cirrhotogenic factors including nutritional deficiency are simultaneously operative. This view might well be supported, but the fact remains that viruses can be regarded as related to certain forms of cirrhosis in as definite a way as the tubercle bacillus may be regarded as related to tuberculosis. It is likely that viruses if they produce progressive cirrhosis do so because they become established in the liver over a long period of time.

(2) *Tropical parasites* have been blamed in the past for cirrhosis of the liver, but their etiological role seems to be far less definitely established than is that of the viruses. Schistosomiasis might be picked out as the tropical parasite for which there is most evidence of a cirrhotogenic action. The fact remains, however, that wherever schistosomiasis is prevalent malnutrition is also prevalent, and there is no satisfactory evidence that any tropical parasite,

and the Gillmans' "cytosterosis" concept. It is felt that the use of the term "haemochromatosis" by Strachan²⁷ and by the Gillmans¹³ for the siderotic cirrhosis of the Bantu people of southern Africa is confusing since the balance of evidence suggests that these cases are quite distinct from classical haemochromatosis.²⁸

Primary liver cancer or primary carcinoma of the liver These terms are used interchangeably and are preferred to "hepatoma" and "hepatocellular carcinoma" in this communication because the former terms are wider and the precise cellular type of carcinoma is not always specified in reports from the areas of prevalence. In all probability, however, the prevalent condition is usually a hepatocellular carcinoma.

Pellagra The term "infantile pellagra" has been specifically rejected in favor of kwashiorkor, and a few words should be said about its use in relation to the prevalent clinical syndrome in adult Bantu subjects in South Africa which is linked by the Gillmans¹³ with the siderotic cirrhosis which they call haemochromatosis. They do not define the "pellagra" they are describing except in the negative sense "We are of the opinion that, unless the dermatosis is present, then the nutritional syndrome should not be regarded as pellagra." They clearly recognize the possibility that pellagrous dermatosis may occur in several different patterns of complex metabolic disturbance resulting from different dietary patterns, but use the term indiscriminately for them all. We see, in Cape Town, pellagra in Cape colored people, of mixed but predominantly non-Bantu blood,⁴ which is indistinguishable from the classical pellagra described in Southern Europe and the Southern U.S.A. We see also "pellagra" in Bantu subjects which is apparently indistinguishable from the "pellagra" described by the Gillmans. In our experience, the disease in these two different races is very different and it is confusing to call them both pellagra. I prefer to call the latter variety "Bantu malnutrition with pellagrous dermatosis." At least it ought to be called "Bantu pellagra" to distinguish it from the classical type. To mention only a few of the differences, pellagra in the Cape colored people is not associated with siderotic cirrhosis nor with any increased susceptibility to primary carcinoma of the liver. The same is true of classical pellagra in Southern Europe and Southern U.S.A. "Bantu pellagrins" in South Africa, on the other hand, have a high incidence of siderotic cirrhosis and primary liver cancer.

Necrosis Experimentally fatty liver cirrhosis and acute necrosis of the liver appear to be quite distinct processes as is evidenced by the division of the proceedings of this conference between these two headings. There has been a tendency among experimentalists in dietary liver damage to confine the term 'necrosis' to the acute necrotic variety instead of using it in its wider meaning of 'death of cells' from any cause. This trend may be useful although it is etymologically dubious. It is necessary, therefore, to emphasize that Brock and Autret in their report used the term in its wide sense and did

fatty liver cirrhosis, but the analogy cannot be pushed too far. This is particularly important in respect to adult "nutritional cirrhosis". In discussing this subject Himsworth¹³ says "massive hepatic necrosis and its sequelae are usually common in tropical and subtropical races subject to malnutrition". The factors, other than diet, which may be involved in the production of tropical cirrhosis are categorized in the text. Some of these are known to cause acute necrosis of the liver.

Postscript

After hearing the discussion at the conference on which this monograph is based, I think it is necessary for me to emphasize that my personal experience of kwashiorkor is limited to the African continent, but includes the material seen in Johannesburg and Pretoria where the syndrome is seen in Bantu children who have usually not been exposed to tropical parasites, and in Cape Town where among Cape colored children there has been no opportunity for exposure to tropical parasites. With regard to the comparison with "American kwashiorkor", I am handicapped by the fact that I have not yet seen the report by Autret and Behar¹. My impression from the published literature and from pictures shown is that American kwashiorkor¹ at least in cities is often more dominated by undernutrition than is the case in what has been defined as "protein malnutrition, especially as exemplified by the pictures in the monograph by Brock and Street". Obviously, there are in all areas all degrees of transition between them malnutrition and undernutrition. Conversations at the conference confirm my impression that cirrhosis and primary carcinoma of the liver are far more prevalent in the American tropical belt than the literature indicates.

References

1. AUTRET C & F TANGUY 1949 Bull med Afr occid franc 6, 99
2. BERMAN C 1952 Primary Carcinoma of the Liver H K Lewis London
3. BIGWOOD E J 1952 See Commission for Technical Cooperation in Africa⁷
4. BROCK J F 1952 The Cape coloured people S African Med J 23 1000
5. BROCK J F 1949 Kwashiorkor in Africa W H O Monograph Series (8) and Bull W H O 6 1
6. CISA FORMATION SYMPOSIUM ON LIVER DISEASE 1951 J and A Churchill London
7. COMMISSION FOR TECHNICAL COOPERATION IN AFRICA SOUTH OF THE SAHARA 1954 Report of Second Conference on Nutrition London England In press
8. DAVIS J N P 1952 Nutrition and Nutritional Diseases Ann Rev Med 3 99
9. DAVIS N C 1954 The macroscopic examination of 29,593 human livers from Central and Northern Brazil Am J Hyg 59 567
10. DEAN R F A 1952 Treatment of kwashiorkor with milk and vegetable proteins Brit Med J 2 791
11. EDENOVIC C M 1952 See Commission for Technical Cooperation in Africa⁷
12. GALT G 1945 Viscerotropic encephalopathy Quoted in Trop Diseases Bull (1946) 43 924
13. GILLMAN J & T GILLMAN 1951 Perspectives in Human Nutrition Grune and Stratton New York
14. GOLDBLATT H 1947 See Macy Conferences on Liver Injury¹⁴
15. GYRGEY P 1953 See Commission for Technical Cooperation in Africa⁷

be used to achieve complete release. Still fresh in our memory is vitamin B₁₂ which not long ago was called "animal protein factor". This monograph contains two further examples of specific nutritional factors which occur strongly bound to protein. Both of these concern the liver. The xanthine oxidase factor which after prolonged attempts at purification and isolation from plant proteins has now been disclosed to be a trace element, molybdenum,¹ and I factor 3 against dietary necrotic liver degeneration which is obtained in concentrated form from casein and which is present even in so-called vitamin free caseins.²

Taking these observations into account and realizing further that not all of the factors protecting against fatty liver and cirrhosis have been identified as yet I think it would be fair to say that one should be reluctant to label Knashnorkor as 'protein deficiency', at least as long as it has not been shown to be cured by pure amino acid mixtures.

References

- 1 H PETERFELD W H & D A RICHERT 1954 The Xanthine Oxidase Factor (Molybdenum) Ann N Y Acad Sci 57 (6) 896
- 2 KNASHNORKOR K 1954 Factors Protecting Against Dietary Necrotic Liver Degeneration Ann N Y Acad Sci 57 (6) 878

THE PATHOLOGY OF DIETARY LIVER DISEASE IN TROPICAL AFRICA

By J N P Davies

Department of Pathology, Makerere College Medical School, and Mulago Hospital, Kampala Uganda

The only form of dietary liver disease in tropical Africa about which it is possible to speak with any precision is the extreme fatty infiltration which is seen in kwashiorkor. In a florid case of this disease (e.g., in a child of about 3 years of age), the liver, while not very much enlarged, is a tawny yellow buff color or, in a very anemic child, may be stone colored, due to intense fat infiltration. In older children, some degree of increased fibrosis of the liver may be appreciated. Not all children with kwashiorkor have such a degree of fatty infiltration, for in some there may be little or no fat.

The changes in the liver in kwashiorkor, as shown by biopsy and autopsy studies, go through a sequence which results in permanent alterations to the structure of the liver. In West Africa, attention has been drawn (Auffret and Tanguy, 1950, Silveira and Jelliffe, 1952) to the presence of gross fatty infiltration of the liver in very young children. Such lesions are not commonly encountered among young children in Uganda and where there is any appreciable degree of fatty change seen in the infant liver, it is usually centrilobular in distribution. So far as my experience goes the livers of newborn African children and of neonates in Uganda do not differ appreciably from the livers of European children. Apart, therefore, from the presence of malaria pigment, which after a while acts as a convenient delimitator of the boundaries between liver lobules, the African liver in those first few months of life, when the child is growing well (Welbourn, 1951), presents no significant change.

fat first at the periphery of the liver lobules. This is always the case in children where there is no precipitating factor, e.g., tuberculosis. In complicated cases the initial fat deposition may be centrilobular or irregularly sited, varying from lobule to lobule. Where this has been recognized by a study of biopsy material, the attention of clinicians has, on several occasions, been drawn to the presence of a hitherto unrecognized infective condition (Davies, 1948, 1950).

Provided there is no such complicating factor, the initial locus of the fat deposition is in the cells at the periphery of the liver lobule, though the cells of the lamina limitans round the portal triads often remain free of fat for a considerable time. As the clinical condition deteriorates the peripheral fat droplets increase in size and the cells down the sinusoids toward the central vein are progressively involved till in a severe case, practically every cell is full of fat and some small fatty microcysts may be seen. FIGURE 1 shows a part of two liver lobules with a portal triad and two central veins with fatty infiltration of the liver cells, the larger lobules of fat being in the outer parts of the lobule. Dark malaria pigment is seen also, collected chiefly at the periphery

With the infiltration of the fat into the liver, certain other changes are noticeable. The lymphocytic infiltration of the sinusoids has been mentioned but these cells and many others become concentrated in the portal triads. The most plentiful cells are lymphocytes, but plasma cells and macrophages and neutrophil and eosinophil polymorphonuclear cells may also be present the latter two types being plentiful in cases complicated by a septic infection ■ ancylostomiasis.

Such notable collections of cells in the portal triads are seen in FIGURE 2. As you can see, there is a considerable degree of fatty infiltration with the most intense fatty deposition in the peripheral cells of the liver lobule. The portal triads are crammed with cells and they are also present to some extent along the periphery of the lobule. The cellular infiltration of the portal triads seems to be one of the persisting alterations in the liver because, except in the early months of life, it ■ very rare to see an African liver which does not contain an excessive number of cells in the portal triads. In addition, comma shaped nuclei are present in fair numbers in the portal triads, and these are probably the nuclei of fibroblasts. They do not seem to occur, in the early stages at least, outside the portal triads.

The third change that takes place is in the reticulum framework in the periphery of the lobule. The fibres thicken and split and reduplicate. This change takes place round the peripheral cells and is most marked at an early stage near the portal triads, but later it ■ apparent round the whole of the periphery of the lobule. The lamina limitans cells are separated by the thickening reticulum and they and the peripheral cells appear to undergo a process of strangulation for some of them disappear with collapse of the reticulum framework. The reticulum undergoes collagenization, so at an early stage, the lobular outline is accentuated not only by the malaria pigment, but also by the reticulum thickening and collagenization. FIGURE 3 shows such fibrotic changes. The liver ■ moderately fatty, the portal triads are being joined one to another by the reticulum thickening and collagenization. This accentuation of the lobular pattern of the liver ■ never so marked in children we have studied that it could be called a cirrhosis and these changes all occur round the lobule periphery. In a few cases, the collagenization is so marked that all the portal triads are linked together by fine strands of fibrous tissue, more reminiscent of the liver of a pig than a normal human liver. In his studies of the fatty liver in cases with tuberculosis, Saphir (1929) remarked on the development in a small percentage of his cases of such a true unilobular type of fibrosis.

In time, should the child survive even without treatment by a high protein diet, the fat will diminish and slowly disappear from the liver even without any marked clinical improvement, and it can be removed more speedily by feeding a high protein diet. Under both circumstances, the fat diminishes and disappears first from the centrilobular regions and last of all from the peripheral cells. As it leaves the fibrosis often seems to increase and become more apparent. The cells after the fat has left, exhibit the same hypocytoplasmic appearance as before it appeared. Though the reticulum thickening and fibrosis seem to start with the fatty infiltration, the relationship ■ certainly not simple. It ■ a time relationship rather than a causal relationship.

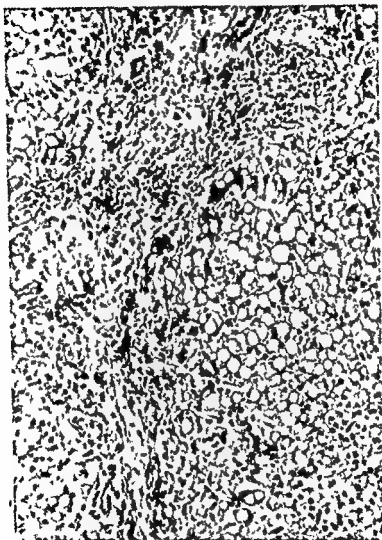


FIG. 2. Low power photomicrograph showing extensive necrosis and cell infiltration at the periphery of the lobules, where the fat infiltration is maximal. H. & E. stain. $\times 210$.

Sometimes there is severe fat infiltration with minimal fibrosis, sometimes marked fibrosis with little fat infiltration, and the cause of the fibrosis awaits clarification, particularly as Waterloo (1951) has shown in West Africa that a similar type of fibrosis can develop in the liver without fatty infiltration. Our experience leads us to suppose that there are at least two sets of causal factors



FIGURE 3. Liver of child with kwashiorkor showing gross fatty infiltrations. The early periportal spreading from the portal tract can be seen. H & E stain. $\times 210$.

operating—one leading to the fatty infiltration, the other to the fibrosis. The net result is, however, that the vast majority of Africans, both adult and child, suffer from the same disease, and the same pathological changes.



FIGURE 4. Biopsy specimen of African child's liver showing stellate fibrosis of the portal triad extending to delineate the lobular boundaries. Retained in situ. $\times 150$.

the next along the boundary of the lobule. FIGURE 4 taken from a liver biopsy shows this lesion which I call a 'stellate fibrosis'. The smooth outline of the portal triad and the lamina limitans are disrupted by these fibrous scars in the vast majority of African livers. I once suggested (Davies 1948) that these lesions were the 'hallmarks' of kwashiorkor. In Uganda, they are not

found before the weaning period and they are almost universal afterward so that some changes take place in this period that mark the period if not the disease

It would, I think, be most unwise for me to speculate on the possible causes of the fibrosis. I do not think the fibrosis is a result of the fatty change but whatever the cause the stellate fibrosis is a persistent lesion and I think it continues in a slow, stealthy fashion to increase in the absence of fat from the liver. Nor have we any very clear ideas on the exact causes of the fatty infiltration except a very strong suspicion that it is somehow linked to the atrophy of the pancreatic acinar cells and the other cells which lose protein by enzyme secretion. The histological evidence we have accumulated suggests that the pancreatic atrophy precedes the liver changes and that the fatty infiltration of the liver is a transient though important episode in the disease. The primary involvement of the pancreas has also been shown by Vegheli (1951). But the precise connections between the pancreatic atrophy and the fatty infiltration of the liver have not been discovered, and to discuss them would take me outside my field.

It is more important to discuss the relationship of the changes in kwashiorkor to disease of the liver in later life. We are most ignorant about the condition of the liver in the later years of African childhood and the earlier years of adolescence. But during this period, and indeed for the rest of his life the African is eating a diet qualitatively little better than that on which he developed kwashiorkor. A diet low in protein, especially animal protein, is high in carbohydrate is all too common. Yet cirrhosis of the liver is rarely seen in childhood in Uganda nor in the early years of adolescence. Puberty with its growth spurt does not bring out a fresh crop of kwashiorkor cases as we do not know why this is so. Perhaps puberty in Africans is more delayed and the growth spurt not so marked. We know that the onset of menstruation is delayed. Moreover, we must remember that the endocrine changes in

much early work on this disease was largely devoted to the study of adult cases. The atrophy of the pancreas and of the intestines is just as marked and the is the same fatty infiltration of the liver though this is not so extreme. The major difference lies in the much greater amount of fibrous tissue present in the liver, a considerable increase in the stellate fibrosis. In some cases regular strands of fibrous tissue cut off groups of cells at the periphery of the liver lobules. Both clinically and pathologically, some cases merge into Laennec cirrhosis but the connection between these states remains obscure. The gross fatty infiltration seen in children is not very frequently encountered in adults and we have no evidence to suggest that our cases of Laennec cirrhosis have gone through a long continued stage of fatty infiltration in adolescence. Iron pigment is not present in unusual amounts in our cases and the only pigment seen is a brown perinuclear pigmentation apparently due to a lipochrome substance.

Laennec cirrhosis is quite frequently encountered in Africans. Among 2/1

autopsies there were 115 cases as well as 74 cases of postnecrotic scarring. Analysis of these cases has shown (Davies 1952) that, contrary to expectations,

and probably to protein malnutrition.

I shall, if I may, conclude this brief presentation by summarizing our main problems as I see them. The frequency and importance of kwashiorkor is now well recognized. The basic lesion seems to be a selective atrophy of the enzyme secreting glands affecting the pancreas, the small intestines, and the salivary and lactimal glands. We do not know the precise cause of this atrophy. At an early stage in the disease there is an intense but transient fatty infiltration of the liver, the fat droplets occurring first in the peripheral cells of the liver lobule, which are the last to be freed of fat. We do not know the cause of this fat infiltration, its relation to the preceding enzyme cell atrophy, or the factors that enhance its removal from the liver. With the fatty infiltration there occurs a flooding of the portal triads and to a lesser extent the sinusoids with inflammatory cells, the lymphocytes being especially prominent. We do not know what they are doing. There is also the reticular thickening and the fibrotic process near the portal triads and around the lobule periphery. The cause of this awaits elucidation. We do not know how this continues to progress in the absence of fat from the liver, the significance of this progression, its extent, or its relation to Laennec cirrhosis. We do not know the cause of the great frequency of Laennec cirrhosis, but we do not think that it is a sequel of infective hepatitis. We also do not know the causal factors of carcinoma of the liver although we suspect them to be endocrinal. Malnutrition, liver disease, and hormonal alterations seem to be fundamental to our understanding of disease in Africans. They are being investigated by only a few widely scattered scientists.

References

- AUFRET C & F TANGUY 1950. *Bull. med. Afr. occid. franc.* 6: 183.
 DAVIES J N P 1948. *Lancet* 1: 317.
 DAVIES J N P 1950. *Liver Injury Trans. 9th Conf. Josiah Macy Jr. Foundation*.
 DAVIES J N P. *W. African Med. J.* 1952 1: 141.
 SAPHIR O 1929. *Arch. Path.* 7: 1026.
 SILVEIRA W D & D H JELLIFFE 1952. *J. Trop. Med. Hyg.* 55: 73.
 VEGHELYI P V 1950. *Ann. Paediat.* 175: 349.
 WATERLOW J C 1951. *Personal communication*.
 WELBOURN H F 1951. *W. African Med. J.* 28: 428.

CLINICAL ASPECTS OF THE TREATMENT OF KWASHIORKOR

By H C Trowell

Mulago Hospital and Makerere College Medical School Kampala, Uganda

TERMINOLOGY AND ETIOLOGY

Although this paper discusses the clinical aspects of kwashiorkor, it is of all necessary to discuss briefly etiology, for this determines the approach to the problem of therapy. There is now general agreement that the clinical and pathological disease process, called kwashiorkor, is very common in certain tropical countries. This condition occurs mainly in young children and follows the prolonged consumption of diets which have lacked variety, in which the proportion of protein, especially that of animal origin, has been low, whereas the proportion of carbohydrate has been high. These diets have had rather a low protein-calorie ratio and the animal protein-calorie ratio has always been very low. It is considered that this unbalanced diet was usually consumed in amounts sufficient to satisfy the child's hunger, but more information is desired on this important point. At a certain stage in the illness, appetite decreases and anorexia begins, so that a decreased intake of this unbalanced diet often then occurs (Welbourn, 1953). At this stage of the illness, there is also impaired digestion and utilization of all foods. The intake of fats is usually low. Children who develop kwashiorkor have been receiving little or no milk, either of human or animal origin, and very little protein of animal origin. The condition is therefore treated, cured, and prevented by giving diets rich in protein. I should also make it clear that certain of my colleagues in Uganda consider that older children and adults may occasionally suffer from a similar or an allied disorder, but this paper is confined to a study of the disease in young children, among whom the condition is far more common because of their high requirements for protein and allied substances.

Kwashiorkor is one member of a big group of diseases referred to as the diseases of protein malnutrition. It would appear unwise to speak of kwashiorkor as merely "protein deficiency" for there are many varieties of protein deficiency even among children. For instance, one type of protein deficiency occurs in nephrosis and it is quite distinct from kwashiorkor. In addition, it is quite uncertain how far calorie intake, total protein intake, one or more amino acids, or vitamins are responsible as well as other substances known or unknown but concerned with animal protein or with protein metabolism. It is also not known why an adequate supply of human breast milk, which has rather the low protein-calorie ratio of 1.5 gm. to 100 calories is so highly protective against kwashiorkor (Gyorgy, 1952).

A noncommittal term such as kwashiorkor (Williams, 1933), is therefore preferred. In many areas of Africa in which there is little cows' milk and protein-rich cereals the peasants have noticed that healthy, breast-fed babies often develop a serious illness shortly after complete weaning. In the tropical countries as in Europe until Elizabethan times breast-feeding

* Thanks are due to the Director of Medical Services, Uganda, for permission to publish this paper.

continue for two or three years and often with considerable benefit. This was often possible in Africa under the conditions of marital life which were a part of polygamy, but conditions and customs are changing rapidly. If a

people of the Gold Coast, among whom Doctor Cicely Williams was working when she published her accounts of this disease (1933). Other tribes have other names and about fifty different terms have been employed by different doctors. These names often refer unfortunately to multiple deficiencies, possibly those of the vitamins, and these terms have crept into the literature, especially that of South America. Yet treating these cases mainly by multiple-vitamin therapy often results in fatalities.

Recently it has become clear that Czerny & Keller (1906) were describing an identical condition in Germany under the name of *Mehlnahrschaden*. Their description of the disease, however, was very incomplete. No changes were noted in the pigment of the hair and the pancreatic lesion was not described so that subsequent workers, such as Véghelyi (1948, 1950), who almost re-discovered the condition in Hungary and called it "nutritional edema," were unable to identify this disease with *Mehlnahrschaden*. The old and correct teaching of the German pediatricians was kept alive and developed in Italy, where Frontali (1952) has confirmed the presence of the pancreatic lesion and the hair changes in this disease (called "starchy food dystrophy" in that country). This disease is present in nearly 5 per cent of children in Southern Italy. The presence of the characteristic 'enamel paint dermatosis' and "flexural fissures" of kwashiorkor has however not been described in any case of *Mehlnahrschaden*, or "starchy food dystrophy."

In the literature of the United States a few cases of kwashiorkor probably have been described, such as that of McEnergy (1933). It is difficult to evaluate the more numerous reports on the cases of "nutritional edema" seen in young children in the United States and in other countries. In cases of "nutritional edema" in infancy, there has not been described the dermatosis, the fissures, the dyspigmentation, the pancreatic lesion, or the high mortality, which are characteristic features of kwashiorkor. "Nutritional edema" was ascribed at one time to protein deficiency and it is probable that many cases of this disease, especially among young children, were cases of kwashiorkor, and were not cases of hunger edema due to calorie deficiency, or *beri beri* due to thiamine deficiency, all three of which are different varieties of "nutritional edema." The latter is not a suitable term for kwashiorkor, for some severe cases show no edema and mild cases of kwashiorkor never show any edema.

probably very common in many tropical countries. It is, however, difficult to define (FIGURE 1). Thus, Welbourn (1953) has found signs suggestive of mild kwashiorkor in 46 per cent of the children attending child welfare clinics near Kampala, Uganda. Wherever possible, treatment and prevention of

the milder disease should be by vegetable protein, for this is cheaper and more plentiful

THE CLINICAL PICTURE OF A TYPICAL CASE OF SEVERE KWASHIORKOR

This is so distinctive (Trowell, Davies, & Dean, 1952) that a diagnosis can usually be made as soon as the case is seen (FIGURES 2 and 3). The child is seriously ill and is usually from 9 to 36 months of age, although typical cases can occur outside this range. The weight and height are always very subnormal. Mental apathy is always present and peevishness on being disturbed is usually noted. The appetite in advanced cases is always very poor. The abdomen is usually distended. Undigested food is passed in bulky or loose stools, which are usually frequent. Diarrhea may be very severe or absent and depends on the food eaten. Subcutaneous fat is often well preserved during the early stages but is lost later if life is prolonged. The muscles waste early and severely in this disorder. Edema is usually present, but may be absent or slight in cases otherwise typical. Edema tends to be generalized, the feet and face being involved early. Effusions into serous sacs are absent or slight.

some change in its texture, becoming soft and straight. It is easily shed or pulled out of the scalp. Some change and loss of hair pigment is usually but not always, seen, but this change may only be indisputable in those whose hair previously was black. Dyspigmentation, however, occurs in all races whatever the color of the hair. Some change in skin pigmentation may occur. Generalized hypopigmentation especially obvious around the mouth may be seen in Africans. Races with a white skin usually show no change but generalized hyperpigmentation has been occasionally described.

The various eruptions on the skin are complex and should not attract too much attention. Like the changes in the mucous membranes they are essentially variable. Usually they are only seen as a terminal condition in very advanced cases. They are not present in all cases even at death. The only dermatosis which, in my opinion, is peculiar to kwashiorkor is best described as "enamel paint dermatosis" (FIGURE 4), or "erosive dermatosis" since, although originally likened to the "crazy pavement" of an old English garden path, this is not a happy comparison and the latter term has been used to describe several different skin lesions. "Enamel paint dermatosis" appears almost exclusively on areas exposed to much pressure, especially if this is combined with moisture, any discharge, or the sweat which is common in the tropics. This dermatosis has a definite evolution. At first, small purple stains darken rapidly into hyperpigmented, raised, burnished plaques and these appear first in the inguinal and nappy areas and over the bony prominences of the legs, trunk, arms and head (FIGURE 5). These plaques have a sharply defined linear edge. They enlarge rapidly and coalesce, then proceed to loosen as with some underlying moisture and peel thus disclosing pale, eroded, moist areas of raw skin. These plaques never appear in response to sunlight, although in many respects they resemble the eruption seen in certain



FIGURE 1



FIGURE 2



FIGURE 3

FIG. 1 (left) African chills with kwashiorkor underweight for multistatist much loss of weight and bluish tinge to the skin after many weeks. FIG. 2 (center) African chills with kwashiorkor showing wasting and edema. FIG. 3 (right) African chills with kwashiorkor showing wasting and edema.

cases of acute pellagra. At times, erosions may occur in any of these areas with little in the way of preceding plaques. These erosions are easily dismissed as areas of intertrigo, which indeed they are. At the same time, in a few advanced cases, very deep fissures occur in the flexures, especially if sustained flexion of a limb causes pressure on the crease in the skin. "Flexural fissures," therefore, appear in the armpit, front of the elbow, back of the knee, and behind the pinna (FIGURE 6), and at the angle of the mouth, where they resemble angular stomatitis, but they are deep fissures which bleed and never appear sodden. "Enamel paint dermatosis," "erosive dermatosis," and "flexural fissures" have not been described, as far as I am aware, in any other disease. All commence healing within a few days, if not hours, after giving skim milk protein.

Large areas of glazed and "crackled skin" may occur in areas exposed to irritation and to dryness. This skin shows little tendency to desquamate spontaneously in large plaques unless the condition is very extensive and is subsequently well treated. This condition, often called "mosaic skin" or "crackled skin," would appear to be largely a nonspecific sign and may occur in many states of undernutrition and responds to an increase in the diet. In my opinion, these conditions tend to be more severe in any disease of protein malnutrition than in states of undernutrition.

In addition, a pellagrous eruption, even with vesiculation, may occur at the periphery of the limbs on areas exposed to sunlight and to wind. It is most common in children taking much maize, but it can occur on other diets. This dermatosis only occurs as a terminal manifestation a few days or weeks before death.

(Gillman & Gillman 1951), or in Italy (Frontali 1952), where it is accompanied by hyperpigmentation of the exposed areas. The latter investigator has shown that this dermatosis responds rapidly to small doses (large amounts are very dangerous) of niacin and only very slowly to skim milk powder, which, however, contains small amounts of tryptophan and of the Vitamin B complex. This explains why cases at one time were considered to suffer from "infantile pellagra." It must also be admitted that transitional conditions between "enamel paint dermatosis," pellagrous dermatosis and "mosaic skin" are frequently seen. These three skin conditions appear to be allied in many ways. They all appear in response to some external injurious agent. They tend to be hyperpigmented. They all tend to desquamate, although to different degrees and at different rates.

It is not for me, as a clinician, to say much about the stellate fibrosis which in Uganda affects the portal tracts and appears first in certain cases after the fatty liver of acute kwashiorkor in childhood. This is described by my colleague Professor J. N. P. Davies in this monograph (page 714). One thing is certain and that is that this stellate fibrosis very slowly increases during late childhood, adolescence and adult life while the patients show no signs of severe kwashiorkor but taken as a group they continue to receive the same diets with a low protein caloric ratio. The diets do not differ from the



FIGURE 4



FIGURE 5



FIGURE 6

FIGURE 4 (above) African child with severe kwashiorkor

FIGURE 5 (below) African child with severe kwashiorkor

taken by infants. It is only the requirements which have altered. In my opinion, these persons often continue to show signs suggestive of mild kwashiorkor, that is to say, they are underweight and have dyspigmented hair and a slightly low serum albumin and a high gamma globulin. Frank cirrhosis of the liver, unassociated with alcoholism or manifest tropical disease, is common in Uganda, even in young adults in the third, fourth, and fifth decades. In my opinion, progressive stellate fibrosis often leads to frank cirrhosis in Uganda, and all varieties of transitional forms are seen. Acute necrosis of the liver, in any form, is very rarely seen in children in Uganda. It occurs in adults and may lead apparently to cirrhosis. It is impossible and undesirable to do more than mention necrosis here, largely because necrosis of the liver has been reported as occurring in kwashiorkor. This is extremely doubtful, except possibly a necrosis of individual hepatic cells, and almost no reference can be traced to this in any original communication on kwashiorkor.

It is not proposed to devote any further time to a consideration of the other signs of vitamin deficiency. These are seldom seen except in the terminal states of kwashiorkor, and it should be noted in passing that signs of vitamin deficiency seldom occur in children or adults dying of general undernutrition due to caloric deficiency. Vitamin A deficiency appears to be very common in cases of kwashiorkor in Asia. Riboflavin deficiency is severe on maize diets. Both scurvy and rickets are said to occur, but are rare, especially the latter in most areas. Thiamine deficiency has not been demonstrated in Africa but is considered to be present in some cases in Italy (Frontali, 1952).

It will be noted that, in all this description of the clinical state, no reference has been made to the liver. In many cases of kwashiorkor, the liver does not appear to be enlarged during life, and there is little data to suggest that the degree of enlargement has been assessed in cases coming to autopsy. In Uganda, it is seldom enlarged during life or at postmortem. Abdominal distension may obscure enlargement. On the other hand in certain parts of the world, moderate enlargement is often detected during life (Waterlow, 1953; Altmann, 1948) and I have seen cases showing enlargement in Kenya, Sudan, Nigeria, the Gold Coast, and South Africa. If, however, the liver in any severe case of kwashiorkor, whether enlarged or not, is biopsied, marked fatty infiltration is almost always seen. In noninfected cases, this commences first, and disappears last, at the periphery of the lobule, that is, near the portal vessels. Any direct transition from the fatty liver of acute kwashiorkor to frank cirrhosis (meaning gross disorganization of liver architecture by fibrous tissue and nodules of hyperplasia) has never been recorded in any part of the world in kwashiorkor in infancy.

It is perhaps as well to close this description of the clinical picture by noting that the majority of cases of kwashiorkor in tropical regions have some infection present.

Many of these such as the hookworms, deplete the body of proteins and increase the dietary requirements. In the majority of cases, in my opinion, it is very doubtful if these tropical infections play any significant part in producing kwashiorkor and recent work (Welbourn 1953) suggests that, even in the tropics, bouts of respiratory infections are the commonest diseases.

precipitate an attack of severe kwashiorkor. In many cases, these infections

are severe or produce fever, it is essential to treat them while administering suitable dietetic treatment.

This clinical picture would not be complete without reference to the biochemical changes. These are regarded as constant features and it is desirable that communications on this disease should include biochemical data. The most constant changes are a marked reduction in the serum albumin, in all the pancreatic enzymes (Thompson & Trowell, 1952) and in the plasma esterase and plasma lipase (Srinivasan & Patwardhan, 1952), in serum amylase, cholinesterase, and alkaline phosphatase. There are also a reduced blood urea, total cholesterol, and ester cholesterol (Dean, 1952b). Feeding with protein rich diets produces a characteristic biochemical pattern of recovery, which

includes, together with the usual laboratory changes, a fall

THE TREATMENT OF CASES OF SEVERE KWASHIORKOR

The mortality rate in untreated cases with edema and anorexia is almost 100 per cent, and little improvement was effected until pathology and biochemistry had afforded some insight into the mechanisms involved. In the tropics, at one time, these cases were ascribed entirely to some tropical infection or to congenital syphilis, and treatment remained unsuccessful until the diets were also improved.

was erroneously focused on the vitamins especially those of the Vitamin B complex and on liver extracts. No serious advances were made in treatment until pathology (Davies 1948) afforded some insight into the difficulty of digesting food, and experimental work, largely in the United States and in Europe, focused attention on the fatty liver. Although the use of known hypotrophic factors has on the whole proved disappointing, yet workers in the tropics felt that this disease was probably an aspect of protein malnutrition. The detection of the pancreatic lesion, of the reduction in their enzymes and of the changes in the small intestine have all helped to explain the difficulty in digesting food.

Treatment remains difficult although the mortality has fallen to below 10 per cent in uninfected cases. Deaths are usually due to collapse soon after admission or very serious infections, such as a fulminant pneumonia or tuberculosis. The extreme anorexia, the difficulty in digesting food, an intolerance to all fats and to many carbohydrates and sugars including glucose and lactose makes treatment difficult. Skim milk powder or, in the most severe cases,

calcium caseinate (a more purified form of skim milk protein and one which contains little lactose), combined with a suitable form of carbohydrate, remains the treatment of choice and may initially be given by tube-feeding. The principles and methods of treatment have been set forward recently by Dean (1952a). It is important to start treatment of the infant somewhat slowly with about 20 gm daily of protein, derived from about 60 gm of ordinary skim milk powder. For calories nothing appears better than fully ripe bananas, which are well tolerated as in celiac disease. After a few days or weeks depending largely on the character of the stools, the diet can be increased very slowly, until eventually children of two years are receiving about 5 gm of protein per kg of body weight (after loss of the edema) and over 100 calories per kg. That is to say the diet has about 5 gm protein per 100 calories almost all the protein, at least at first, being derived from animal sources. Dean (1952a) has reported cures with vegetable protein supplemented by vitamins, including B₁₂, but in advanced cases skim milk protein remains the most efficient form of treatment.

Within a few days, a definite response occurs. The general condition improves, diuresis occurs, "enamel paint" is improved but tend to remain bulky and contain undigested food, and diarrhea may recur if much carbohydrate or fat is taken and after every successive addition to the diet. The pancreatic enzymes increase, the serum albumin rises very quickly, the gamma globulins increase and tend to remain high for a very long period of time (Anderson & Altmann, 1951).

As assessed by serial liver biopsy, fat starts to disappear rather slowly from the center of the hepatic lobules, that is around the central vein, but even after 4 to 8 weeks of treatment, a certain amount of fat is often still visible at the periphery of the lobules. All these and other changes are due to liver extracts all exert a lipotropic effect, whether any of them is essential is not known.

It is doubtful if special supplements of methionine or choline have been demonstrated to accelerate this slow lipotropic action of whole animal protein although, in Chile, Meneghello and Niemeyer (1950) considered that some acceleration occurred in infants given 5 gm of choline hydrochloride daily, in addition to 3.5 gm milk protein per kg of body weight.

It is but natural that it should be desired to study the effect of other nutrients, such as individual amino acids and vitamin B₁₂ but this is very difficult if not dangerous, in severe cases of kwashiorkor for, unless they receive skim milk protein they soon die. It is, therefore, essential to take cases that are less critically ill, prescribe a diet which will prevent further deterioration but which will maintain them in almost a stationary condition during a test period of 10 to 20 days and then to add the single nutriment, the effect of which it is desired to study. In actual practice, this is difficult and dangerous. Observations so far have not demonstrated that supplements of methionine aid recovery. No other amino acid has been tried by more than one observer. No clinical response to vitamin B₁₂ has been unequivocally demonstrated.

The administration of this vitamin even parenterally does not improve the anemia which is usually normocytic but may be slightly macrocytic. The bone marrow is probably very seldom megaloblastic as in pernicious anemia. It is usually normoblastic or macronormoblastic. The anemia does not respond to folic acid or to refined liver extracts but it sometimes improves slowly while receiving iron. Anemia is seldom severe unless some infection is also present. It is probable that the anemia is due to protein deficiency and resembles that present in cases of massive albuminuria.

A marked feature of many cases of severe kwashiorkor is that progress is almost always slow and many months often elapse before the child is normal in every respect. No reason is known which will explain the very slow gain of weight usually encountered during the later months of treatment. In this respect the recovery of cases of kwashiorkor appears fundamentally different from that seen in children of the same age who are recovering from a period of



FIG. 1. Congenitally kwashiorkor showing pot belly, thin limbs, and pale hair.

undernutrition and caloric deficiency. Appetite is apt to remain indiffer-
 ent and diarrhea is easily provoked in all cases of kwashiorkor. The failure
 to gain weight rapidly has not so far been explained by any infection, or
 irreversible change in the pathology or biochemistry. Probably more at-
 tention should be paid to the intake, the digestion and utilization of food, since
 it is probable that the first organ to suffer and possibly the last to recover is
 the gastrointestinal tract. The pancreas and liver are possibly affected later.
 This sequence reflects the high protein requirements of these organs. Clinically
 these convalescent children have swollen abdomens and thin limbs (figures
 as two American doctors noted (Payne & Payne, 1927) when they described
 kwashiorkor in the New World, but unfortunately for them, they suggested
 no satisfactory title and called the complaint "Edema disease of Haiti."

In conclusion, I should like to state that, if I were asked what clinical
 signs are of the greatest significance in kwashiorkor, I would answer, without hesi-
 tation, "Appetite and interest." In the early decrease of appetite, which is
 almost abolished in all cases of severe kwashiorkor, this disease differs fun-
 damentally from undernutrition on diets normal in the proportion of the va-
 rious nutrients, but reduced in the total amount. In kwashiorkor, there is
 no desire for food and little interest in life. These are the stigmata of kwashi-
 ork. Is this a response to the fatty liver or, as I suspect, due to some change in
 the functions of the hypothalamus, together with its numerous nervous connec-
 tions and subject to various forms of chemical control? Why should appetite
 increase possibly at an early stage on diets having a low protein caloric ratio?
 How does skim milk powder stimulate hunger in a child dying of kwashi-
 ork? Eventually, it stimulates interest in life and, when a child with kwashi-
 ork smiles, he is well on the way to recovery.

Summary

In recent years a disease process has been described in young infants
 common in tropical countries and whenever the diet of a young child con-
 tains a low protein caloric ratio (especially if the proportion of animal protein is
 low and that of carbohydrate is high). The disease is considered to be identical
 with Mehlirrschaden and many cases have been described as "nutritional edema."
 The latter term describes two or more disease processes: firstly, undernutri-
 tion terminating as hunger edema in which the diet although balanced in its
 proportions is small in amount and deficient mainly in calories; secondly,
 kwashiorkor produced usually by a diet high in carbohydrate and low in
 protein, in amount adequate to satisfy caloric requirements but in which
 fat accumulates at the periphery of the liver lobule and in the
 parts of the lobule. The liver in kwashiorkor is characterized by a
 fatty liver but often by a biochemical picture which is not
 recovery.

The clinical picture of cases of kwashiorkor or in children
 This is very characteristic although the biochemical picture varies
 deficiency may appear. At an early stage, it is characterized by
 organs which are the highest protein requirement organs.

atrophic changes and loss of secretions by the small intestine pancreas and fatty changes in the liver

These features all respond to protein rich diets especially milk protein and animal protein, and it is doubtful if any specific response has been demonstrated to methionine, choline vitamin B₁₂, or folic acid There is uncertainty concerning the mechanisms and the factors which are involved Animal protein especially milk is still the best treatment of advanced cases but prevention and the cure of the more numerous mild cases must be in an increased intake of vegetable protein fortified possibly by factors unidentified at present

Apathy and anorexia are the stigmata of kwashiorkor and separate this disease process from that seen in undernutrition on a diet normal in its proportions but reduced in its amount, thus producing a disease process in which caloric deficiency dominates the response of the body

References

- ALTMANN A 1948 Clin Proc (Cape Town) 7 32
 ANDERSON C G & A ALTMANN 1951 Lancet 1 202
 CZERNY A & A KELLER 1906 Das Kindes Ernährung Ernährungsstörungen u Ernährungstherapie 2nd ed 1928 Leipzig
 DAVIES J N I 1948 Lancet 1 317
 DAVIES J N I 1952 Ann Rev Med 3 99
 DEAN R I A 1952a Brit Med J 2 791
 Commission
 press
 ommission for
 New York
 Am J Diseases Children 90
 11 7 73
 JHANSAN I N & A JALABIAN 1952 Lancet 2 864
 THOMPSON M D & H C TROWELL 1952 Lancet 1 1031
 TROWELL H C 1949 Trans Roy Soc Trop Med Hyg 42 417
 TROWELL H C J N P DAVIES & R F A DEAN 1952 Brit Med J 2 98
 VEGHELVI I A 1948 Acta Paediat 36 123 493
 VEGHELVI P A 1950 Ann Paediat 175 349
 Med
 Univ

VITAMIN E AND CAROTENOIDS IN THE BLOOD PLASMA IN KWASHIORKOR

By H C Trowell, T Moore, and I M Sharman

Mulago Hospital and Makerere College Medical School Kampala Uganda Dunn Nutritional Laboratory University of Cambridge, and Medical Research Council Cambridge, England

Deficiency of protein has been generally accepted as one of the main factors in the development of kwashiorkor. Other dietary deficiencies which might aggravate the want of protein therefore deserve attention. Experiments with animals have clearly demonstrated that their ability to subsist on diets low in protein is influenced by the allowance of vitamin E. Thus Dam¹ found that young rats which were given a diet grossly deficient in protein survived longer when they were dosed with vitamin E than when they were left undosed. Various other workers confirmed the beneficial effects of vitamin E in rats given diets low in protein both upon the body weight and in preventing dental abnormalities.^{2, 3, 4, 5} In view of the severe injury to the liver in kwashiorkor special interest may be attached to the finding of Schwarz⁶ and others^{7, 8} that vitamin E protects rats against the hepatic necrosis which is otherwise induced by protein deficiency.

It seemed desirable, therefore, to try to obtain evidence on the vitamin E status of patients with kwashiorkor by estimations made on the blood plasma. Specimens were sent by air from Uganda to Cambridge, mostly in 1950 but a few in 1951. The plasma was shaken up with ethanol and light petroleum and total carotenoids were estimated by their yellow color in the extract separated after centrifuging. The extract was then evaporated, and the residue was redissolved in hot ethanol. Cholesterol and other sparingly soluble substances were removed by freezing and again centrifuging and "total reducing substances" were estimated in the supernatant solution with $\alpha\alpha'$ -dipyridyl and ferric chloride. "vitamin E" was measured as the difference between the total reducing substances and the correction appropriate for the carotenoid. For purposes of comparison, estimations were also made upon specimens of plasma from healthy male African adults and from a few healthy infants. Specimens were also taken from adults suffering from pyomyositis, which is a disease characterized by degeneration of the voluntary muscles and the formation of abscesses.

From TABLE 1 it will be seen that lower levels were found for vitamin E in kwashiorkor (average 0.67 mg per 100 ml) than in healthy men (average 0.92

The reduction of carotenoids 100 ml as compared with more noticeable. Several red quite colorless to the hues for healthy subjects

of the specimens of plasma gave extracts w eye. The levels in pyo- sitis also fell be both in vitamin E and carotenoids

From these results it appears either that the carotenoids or that the absorption from the diet is impaired by

lack of vitamin E and/or inefficient utilization of

TABLE 1

	No. of cases	Av age	Carotene μg 100 mL		Tocopherol mgm. 1000 mL	
			Range	Av	Range	Av
Kwashiorkor infants	12	2 $\frac{1}{2}$	4-54	23	0.35-1.06	0.67
Normal African infants	4	2 $\frac{1}{2}$	46-142	107	0.85-1.40	1.10
Normal African adult males	10		49-167	99	0.58-1.37	0.92
Pyomyositis in adults	17		13-89	38	0.34-1.38	0.80

low values for the vitamin L has already been found by Darby *et al*⁸ in pa-

disease, cirrhosis of the liver, sprue, celiac disease, and diarrhea. All patients with these diseases had their lowest readings in repeated observations within the range of 0.06-0.25 mgm. The values found in kwashiorkor, therefore, may seem no lower than might be expected in a disease which is characterized by damage to the liver and general impairment of the absorption of food. It is perhaps surprising that the ranges observed did not differ more widely from those found in pyomyositis—a disease in which injuries to the liver are less prominent than in kwashiorkor, but in which a suspicion of vitamin E deficiency might possibly be suspected from the muscular degeneration. Presumably the frequency of fever influenced the levels in this disease.

Even if we regard the reductions in vitamin E and carotene mainly as sec-

servations in a case of sprue. It cannot be ruled out, therefore, that a secondary deficiency of vitamin F may be included among the numerous complications and aggravations which are involved in the progress of kwashiorkor. Our very low values for carotene suggest further that the vitamin A status may be endangered when the reserves of the preformed vitamin which are presumably stored during lactation have been used up.

Summary

Low values for vitamin F and carotenoids, probably related to impaired powers of absorption, were found in the blood plasma of African infants suffering from kwashiorkor.

The possibility of the occurrence of secondary deficiencies or subnormalities of vitamins F and A as complications to kwashiorkor should not be overlooked.

References

1. DARBY, H. 1944. *Proc Soc Exptl Biol Med* 55: 55.
2. VICTOR, J. E. & A. M. LARSEN. 1945. *J Exptl Med* 131: 375.
3. HOVE, L. L. 1946. *Proc Soc Exptl Biol Med* 63: 508.

ury Josiah Macy Jr. Foundation

- 8 HINSWORTH H P & O LINDAN 1949 Nature 163 30
- 9 DARBY W J M E FERGUSON R H FURMAN J M LEMLEY C T BALL & G L MENEELY 1949 Ann N Y Acad Sci 44 328
- 10 FILER L J S W WRIGHT M P MANNING & K E MASON 1951 Pediatrics 4 328
- 11 PAPPENHEIMER A M & J VICTOR 1946 Am J Path 44 395
- 12 TVERDY G A L FROELICH & H FIERENS 1949 Acta Gastro Enterologica Belg 11 221

ASPECTS OF NUTRITIONAL LIVER DISEASE—HUMAN AND EXPERIMENTAL

By Joseph Gillman and Christine Gilbert

Departments of Physiology and Anatomy, University of the Witwatersrand, Transvaal, and the Nutrition Research Unit of the Council for Scientific and Industrial Research and of the University of the Witwatersrand, Transvaal

In this communication we propose, first to examine the relationships of hepatic fibrosis to antecedent fatty change and to necrosis of the liver in malnourished human beings and in animals fed various diets and, secondly, to

The Fatty Liver and Hepatic Fibrosis

A fatty change can be found in livers of malnourished infants and children admitted to hospital during an acute nutritional breakdown (Normet, 1937, Bablet and Normet, 1937, Gillman *et al.*, 1945, 1951, Waterlow, 1948, Van der Sar, 1951, Oomen, 1951, Altmann, 1948). By serial biopsy examination, we

Six monthly biopsies of the livers of infants and children who have left hospital after recovery from an acute nutritional breakdown show no evidence

infant and child pellagrins admitted to hospital, we arrived at the conclusion that although fatty livers may be associated with many diseases fatty change is more frequent and is likely to be more severe in African infants admitted to hospital with nutritional edema or infantile pellagra than in children of the general population

The fatty liver in infants suffering from nutritional edema and pellagra is therefore of relatively acute onset and in a sense is similar to the intensely fatty livers observed in well nourished children dying four to eight days after an acute attack of "summer diarrhoea" or after severe burns. Like the other events associated with the acute episode, a fatty liver is an expression of a relatively sudden and profound disorder of metabolism occurring in a malnourished infant (Gillman and Gillman, 1951)

One question now arises whether the fatty liver in any way influences the development of fibrosis. At the outset, we may say that portal fibrosis, or more than usual amounts of fibrous tissue, in the liver occur frequently in

adult Africans (Gillman and Gillman, 1951) It now remains to attempt a correlation between the frequency of hepatic fibrosis and steatosis

Twenty five of 261 African adults (i.e., 9.5 per cent) dying accidentally showed gross portal fibrosis as compared with 11.9 per cent of 177 adult African pellagrins admitted to hospital. In the general population the incidence of the fatty liver in Africans dying from accidental causes was 2.7 per cent.

In the livers of 79 adult females over the age of twenty years admitted to hospital during an attack of pellagra, 66 (86 per cent) contained some stainable fat and, of these, the livers were moderately or intensely fatty in 35 females (51 per cent). On the other hand, some fat was detected in the livers of 65 (69 per cent) of 94 adult male pellagrins in our series and, of these livers 33 (50 per cent) contained moderate or abundant amounts of fat. Apart from demonstrating the greater incidence of fatty change in females this analysis reveals that a fatty liver occurs much more frequently in pellagrins than in members of the general population.

We are led to conclude that there is no *prima facie* case for claiming the existence of a direct causal relation between the fatty liver and hepatic fibrosis in the malnourished African. The evidence adduced in this connection is as follows: (1) The fatty change in the livers of infant and adult Africans is of relatively acute onset, (2) After the acute episode the fat usually disappears from the liver, (3) Sampling of the livers of the general population at all ages affords no evidence of a persistent and progressive fatty change, (4) The evidence of hepatic fibrosis in the general population of Africans cannot be correlated positively with the frequency of hepatic steatosis. Taking this evidence into account, we affirm once more that the fatty liver, *per se* in the malnourished infant and adult African suffering an acute nutritional breakdown plays no direct role in promoting the high incidence of liver disease in Johannesburg. Subsequent to our own study Dible (1951) independently arrived at the conclusion that even in London there was little evidence to show that fatty infiltration of the liver was a precursor of hepatic fibrosis. But it has been shown by Dible (1951) and by Gillman and Gillman (1951) that a fibrotic liver can undergo a fatty change in which case it might easily be possible to mistake the sequence of these two reactions and to regard the fatty change as preceding the fibrosis.

The frequent coexistence of fat and fibrosis in the same liver has led to the generally held opinion that long standing fatty change in the livers of animals on various diets is an essential precursor of diffuse hepatic fibrosis (Himsworth, 1947). This view implies that the fat filled enlarged hepatic cell occludes the circulation through the lobule and eventually results in necrosis of the parenchyma. Necrosis in turn is said to stimulate the connective tissue reaction an essential precursor of hepatic fibrosis. It would thus appear that, in the fatty liver as in the necrotic liver to be mentioned below, necrosis is the precursor of hepatic fibrosis.

From our own observations in animals we have demonstrated that certain diets will favor the development of an extensive fatty change which can persist for approximately 300 days without any evidence of fibrosis (Gillman *et al.*, 1953). Since it is possible to disengage the fatty change from fibrosis it fol-

shows that the accumulation of fat and the overgrowth of fibrous tissue in the liver are separate processes which are not necessarily causal. Fibrosis can be disengaged from steatosis. Each is independent of the other, and each requires special conditions if the appropriate reaction is to be set into operation. Nutrition experiments in animals have shown that as a rule, fibrosis only supervenes in fatty liver if the latter is progressive and long standing. Such a relationship we have shown does not exist in malnourished Africans. Although nutrition plays a commanding role in promoting the frequency of fibrosis in the malnourished African it does so in other ways than by exciting an antecedent intense fatty reaction.

We wish to point out that the fatty change in the liver may participate indirectly in the development of hepatic fibrosis. An intensely fatty liver in pellagrins is indicative of an acute metabolic disorder. On each occasion when the liver undergoes a fatty change, there is a katabolic process of greater or lesser severity which has as one of its consequences, irritation of the reticulo-endothelial system. Repeated irritation of the reticulo-endothelial system over many years often beginning in infancy, can, in some circumstances culminate in portal fibrosis.

Necrosis and Hepatic Fibrosis

Acute necrosis of the liver has not been established in infant and adult Africans admitted during an acute nutritional breakdown. It is true that

ing to autopsy. We would like to mention that the administration of thiamine to one of our pellagrins unquestionably precipitated diffuse necrosis of the liver recognized from fragments obtained by puncture biopsy (Gillman and Gillman 1951).

There are other circumstances however, usually those associated with acute anoxia which may contribute to necrosis of the liver (Gillman and Gillman 1948). In the first place the frequent use of open braziers to heat ill ventilated huts and shacks during the winter months leads frequently to carbon monoxide poisoning. In a small series of 25 cases of fatal carbon monoxide poisoning we observed hepatic necrosis in two cases surviving for longer than nine hours. Secondly severe hemorrhage resulting from industrial or other accidents and from assaults during drunken brawls produces severe anoxic reactions in the liver. If such individuals survive for a few days the anoxic reaction may eventually culminate in necrosis. We reported two cases of extensive necrosis of the liver following severe hemorrhage in forty cases of multiple injuries. It must be remembered that many of the cases died within a few hours of the injury but they did show an intense reaction in the form of vacuolated anaplastic cells which as we have shown can go on to necrosis. Similar plasmalike cells apparently precede the necrosis of infective hepatitis as shown experimentally by Kyo Hiri *et al* (1952).

Thirdly, severe infections especially those associated with lobar and broncho-

pneumonia, often precipitate jaundice in the African. In some of these cases coming to autopsy, we have found necrosis of the liver. Fourthly, prior to the introduction of antibiotics, intensive and often indiscriminate use of arsenic, ostensibly for the treatment of syphilis among the Africans, may also contribute to the production of hepatic necrosis. We mention this because of the numerous severe reactions we have observed in Africans receiving arsenic, who were sufficiently indisposed to be admitted to hospital. But not all patients, even though indisposed, seek hospital treatment after arsenic therapy. The untoward reactions experienced are often regarded as part of the treatment. Fifthly, the frequency of tuberculosis among the Africans in Johannesburg and elsewhere in this country is so great that it is regarded as a major health problem. Extensive tuberculosis of the liver is a common finding. Even when less extensive, we have found more than suggestive indication that tuberculosis can contribute to a low grade but widespread destruction of liver tissue.

In this connection, we should like to mention that Straub and Schaberg (1950) have drawn attention to the frequency of hepatitis, allegedly due to a virus, in malnourished Indonesians. Perusal of their protocols disclosed that, in every instance cited, a large or small focus of tuberculosis in the lung was noted. Straub and Schaberg regard the prevalence of hepatic fibrosis in Indonesia as an interaction of two factors, namely, malnutrition and virus infection. While we are not entirely satisfied that Straub and Schaberg have made out a case for virus infection in the Indonesian, nevertheless, in view of the greater vulnerability of the liver to infection in malnourished individuals, it seems to us that the role of the tubercle bacillus, as an etiological agent in the production of hepatic necrosis and fibrosis in malnourished individuals, has not been adequately assessed. Moreover, it is our opinion that not only virus infection and tuberculosis but also parasitic infections, such as schistosomiasis and malaria, as well as other infections, including those responsible for lobar and broncho pneumonia, may contribute collectively, either directly by destruction of liver tissue, or indirectly through the production of severe anoxia, to the greater incidence of necrosis of the liver in a chronically malnourished population.

Although we have seen cases with acute and subacute yellow atrophy of the liver in Africans, it is impossible, in the absence of further laboratory examinations, to incriminate the virus of infective hepatitis. Besides the use of medicinal herbs by Africans has been repeatedly suspected of promoting extensive necrosis of the liver of the type mentioned above.

As in the case of the fatty change, necrosis has been regarded as a precursor of hepatic fibrosis. However, it is important to realize that fibrosis is not an automatic response of the liver to necrosis. Necrosis can be disengaged from fibrosis. Both in man and in animals it has been repeatedly demonstrated that, even after extensive injury to the liver, regeneration may be complete [Dible *et al* (1943), Lucké (1944), Cameron and Karunaratne (1936), Orr (1940), Gullman *et al* (1952 b)]. On the other hand, we have demonstrated in animals that whether or not hepatic fibrosis will supervene in a liver which has undergone extensive necrosis of dietary origin is not only a function of

necrosis but also of the diet. If, after necrosis has declared itself, the animals are still maintained on the deficient diet, recurrent attacks of necrosis are to be expected. In this context, provided the animals survive for any length of time, hepatic fibrosis and even nodular hyperplasia are almost inevitable consequences. On the other hand, if necrosis has declared itself and the animals are then given access to a balanced ration, regeneration of the liver is usually complete and fibrosis is prevented. Moreover, by allowing the animals access alternately, for varying periods to the necrogenic and to the balanced diet, it is possible to produce a variety of pathological reactions in the liver. These can be interpreted as representing a combination of necrosis, regeneration, and fibrosis. Gross distortion of liver structure can be one of the end pictures (Gillman *et al.* 1952, a and b).

It is evident from the latter experiment that, although survival of animals fed a necrogenic diet can be prolonged by occasional feeding for limited periods, of a balanced ration, the liver still remains vulnerable and can still be grossly disorganised on a later occasion. Clearly, the more chronic forms of liver

to differences in the ability of rats to take advantage of the good diet, which is, in turn, determined by the extent of pre-existing liver and other organ damage imposed by the necrogenic diet.

to regeneration in better fed animals. Diet then may serve to increase the frequency of hepatic fibrosis in two ways: firstly, by rendering the liver more vulnerable to noxious agents and especially to the effects of acute anoxia, and secondly, by promoting fibrosis once necrosis has supervened.

One question still remains unanswered. Why is there such variability in liver pathology in different members of the same malnourished population? The answer may be given, in part, in terms of the subtle differences in diet consumed by members of the population, the extent to which each individual may gain access to better food during times of plenty, the presence or absence of various infections including pneumonia, tuberculosis, malaria, schistosomiasis *etc.*, and the extent to which occupation and living conditions expose individuals to severe liver injury.

In addition, the difference in the reactivity of livers of malnourished individuals probably has the same basis as the differences observed in the livers of seemingly well nourished patients contracting infective hepatitis during the same epidemic. Of those suffering from infective hepatitis, some may suffer a mild attack and recover rapidly, others develop hepatic necrosis which heals completely, whereas in still others the necrosis may go on to fibrosis. In these instances, the virus of infective hepatitis merely served to expose underlying differences in metabolism of affected individuals already existing even before the disease was contracted. That is to say, although cases contracting infective hepatitis but reacting differently to the virus had ready access to a good diet and were not showing signs of malnutrition, such individuals were probably utilizing their food in different ways. After all, it is well known that two individuals, consuming similar diets, may put the nutrients to different uses. One individual may increase rapidly in weight and another may remain thin.

In this connection, we may recall the classical experiments of Chaikoff and his associates (Chaikoff *et al* 1938, 1948, Gillman and Chaikoff, 1949) who demonstrated the altered utilization of food in thyroidectomized, hypophysectomized and pancreatectomized dogs. Fatty livers developed in all three animals while consuming a diet rich in lipotropes and adequate to prevent a fatty liver when such diet was fed to normal dogs. However, the fatty liver of the dogs with one or other of the endocrine glands ablated could be prevented by increasing the choline intake in amounts which ordinarily would not be available in an average diet.

By analogy, human subjects suffering from varying degrees of subclinical hypothyroidism or of pancreatic disease will not utilize the same food in the same way as another individual with a normally functioning thyroid or pancreas. Indeed, as revealed in the case of hypothyroidism, nutritional disease may even supervene on a diet adequate to maintain an intact organism in good health (Gillman and Gillman, 1951). If then, two individuals, one in good health, the other suffering from subclinical hypothyroidism, are both consuming the same diet, the diet will in the one case be adequate and in the other, deficient. If these two individuals both develop an attack of infective hepatitis divergences in hepatic reactions are to be expected in the same way as has been shown in the case of the reaction of livers of animals subsisting on different diets and poisoned with the same concentration of chloroform. The divergent reaction of livers to the same stimuli, therefore, is dependent on the prevailing metabolism. The latter is not only a function of the diet consumed, but also of the way the nutrients are utilized. The utilization of food in turn is dependent on the functional integrity of the various organs and tissues and the way these various components of the organism are functionally related (Gillman and Gillman 1951). The difference in liver structure observed in a random sample of a malnourished population, therefore, can be explained on the same basis as mentioned above in the case of infective hepatitis.

In addition however consideration needs to be given to the number of previous nutritional breakdowns, the age at which the first breakdown occurred, the extent of collateral organ damage precipitated by infections, *etc.*, all of

as to the particular economic circumstances of each individual from birth until the time the liver is examined

The search for an understanding of the mechanism underlying the prevalence of hepatic disease and more especially of hepatic fibrosis in a malnourished population demands a wider appreciation of the complex factors involved in a biological reaction. As will be indicated in the final section of this paper, the approach to the problem of nutritional disease is not to be sought only in terms of single factors

Methodological Considerations of Nutritional Investigations

It has become common practice among experimentalists to design diets in such a way that the presence or absence of a specific factor may decide whether or not spontaneous liver disease will emerge, or whether or not the liver will be protected against toxic agents. As a consequence, they have identified

With the exception of cystine, the general trend has been toward accepting liver disease as the consequence of a lack of one or other constituents in the diet. The inclusion of 1 per cent cystine in the diet can lead to extensive hepatic necrosis and hemorrhage within a few days (Earle and Victor, 1941). In this instance, a causal relationship can be established between the contents of the diet and the onset of liver necrosis. Even in those diets where the absence of a particular factor can be shown to be related to a particular pattern of liver reaction, too little consideration has been given to defining the type of metabolism promoted by the diet which in the first instance allowed the particular factor to be all important in determining whether or not the integrity of the liver would be maintained. If the emphasis is placed mainly on the

of choline and cystine (Glynn *et al.*, 1945), or alpha tocopherol (Hingsworth and Lindan 1949, Gyorgy *et al.* 1950) or by antibiotics (Gyorgy, 1951), (2) Necrosis of the liver in rats fed on the low protein diet used by Hove *et al.* (1949) may be prevented by tocopherol but not by methionine, (3) Necrosis of the liver in rats fed potato starch and food yeast (low in proteins) may be prevented by alpha tocopherol or methionine, but not by cystine (Gillman *et al.* 1952a) (4) Diffuse necrosis can occur even on high protein diets lacking vitamin A and fat but containing alpha tocopherol and can be prevented and cured by the addition of vitamin A (Gillman *et al.* 1952a)

It is quite clear that, in these different circumstances the constituents of the diet rather than the intrinsic "needs" of the organism have been collectively responsible for imposing a metabolism which required the further inclusion of

a specific factor in the diet for the prevention of liver disease. Moreover we have demonstrated that in so-called low protein diets factors other than protein can decrease the incidence of hepatic necrosis. Himsforth (1941) wrote: 'The most discordant results were received and it is no exaggeration to say that at one time or another every dietary component save carbohydrate has been indicted (as the cause of liver injury)'. We have since shown that even the nature of the carbohydrate can modify the incidence of necrosis (Gillman *et al.* 1952a).

Clearly then the constituents of the diet play a commanding role in creating the conditions for making a specific nutrient a limiting factor in a nutritional syndrome. If the significance of a particular nutrient is to be assessed it is equally important that the conditions be established which allow the nutrient to occupy such a commanding role in preventing or promoting a disease process of nutritional origin. In support of this opinion we should like to present some of our own observations on the prevention or the facilitation of the fatty liver in rats.

Using maize as the main constituent of the diet a fatty liver is much more easily produced in rats than is hepatic necrosis (TABLE 1). If maize alone is fed to adult rats (TABLE 1, Diet 1), in the absence of any intercurrent infection or interference with food intake consequent on injury to the teeth a fatty change commencing as early as the sixteenth day of the experiment declares itself in the liver.

liver
may be
maize a

tion of other nutrients however it is possible even in young rats to produce consistently a fatty change in the liver (TABLE 1 Diets 7, 8, 10 and 11). The most intensely fatty livers were found in young animals fed Diets 10 and 11 and the least intensely fatty in rats fed Diet 7. If the salt mixture is omitted (Diet 9) the fatty change does not appear in the liver. Apart from congestion no other gross or microscopically demonstrable pathology is evident.

It might be argued according to the views expressed by Handler and Dulbecc (1946) that the absence of the fatty liver in young rats is due to the failure of these animals to grow to any extent. We have observed fatty livers in rats fed 10 per cent brewers' yeast containing an equivalent of 0.5 per cent additional cystine and potato starch even though the growth was scarcely different from that of young rats which failed to develop fatty livers while subsisting on maize alone. Then too we have produced a fatty change in the livers of young rats on a diet consisting of 10 per cent vitamin free casein, 30 per cent corn, 50 per cent sucrose, 4 per cent agar, 4 per cent salt mixture, and 2 per cent cystine together with an appropriate vitamin mixture. The growth of these animals was of the same order as that of rats fed Diet 9 in which the salt mixture was absent. In the latter animals (that is those on Diet 9) neither a fatty liver nor hepatic necrosis was observed. Clearly then neither the fatty change nor necrosis of the liver is necessarily a function of a minimum amount of growth in the conditions of our experiment.

The quality and quantity of proteins in the diet are generally believed

TABLE 1

Diet no.	Male	Glucose, gms	Food yeast	Capsule	%Unmilk powder	Salts	Fat	% capsule A, E, & D	Protein content of diet & per cent	No. of rats	Average initial weight (gm.)	Average maximum weight (gm.)	Per cent of survival (days)	State of the liver
1	100	-	-	-	-	-	-	-	8.1	20	261	244	350-400	Intensely fatty.
2	100	-	-	-	-	-	-	-	8.1	15	34	40	65-100	Atrophic
3	90	-	10	-	-	-	-	-	12.2	5	40	64	58-69	Atrophic
4	95	-	-	-	5	-	-	-	9.1	5	34	85	142-231	No fatty change & focal necrosis.
5	80	-	-	20	-	-	-	-	26.5	5	35	68	62	Congested
6	97	-	-	-	-	3	-	-	7.8	5	32	75	104-163	Mildly fatty.
7	72	-	-	-	-	3	10	-	15.7	5	40	340	391 ^a	Mildly fatty
8	82	-	10	5	-	3	-	+	16.5	5	29	315	214 ^a	All moderately fatty
9	75	-	10	5	-	3	10	+	15.9	5	28	88	65-125	Congested
10	77	-	10	-	-	3	10	+	11.1	5	34	254	238 ^a	Intensely fatty
11	92	-	10	5	-	3	10	+	11.6	5	32	206	241 ^a	Intensely fatty
12	82	-	-	-	5	3	10	+	8.2	5	30	206	255 ^a	Moderately fatty
13	67	-	-	-	5	3	10	+	11.6	5	31	373	243 ^a	No obvious pathology
14	57	67	-	-	20	3	10	+	6.2	5	28	127	215 ^a	No obvious pathology
15	57	-	10	-	20	3	10	+	29.5	5	31	325	234 ^a	No obvious pathology
16	72	-	10	-	5	3	10	+	12.3	5	39	505	254 ^a	Intensely fatty

^a Represents the day on which all rats in the experiment were sacrificed.^b Protein determined as nitrogen.^c Data still alive, liver biopsy at 291 days.

determine whether or not fat will accumulate in the livers of experimen-

that our own investigations reveal the unsettled role of proteins in promoting or preventing the fatty liver

From TABLE 1, it is evident that the fatty liver develops in young rats fed Diet 7 and Diet 11. In heavy weight (adult) rats, maize alone is efficient as a fatty liver producing diet. If we examine the protein content of all these diets (TABLE 1), it is clear that in our experiments the fatty liver was produced at protein concentrations ranging from 8.1 per cent to 15 per cent. In the case of the maize alone (Diet 1) the fatty liver could be prevented or cured by the administration of 50 mg. of methionine per rat per day. This latter observation would seem to indicate that, even in diets with over 15 per cent protein there is still a lack of methionine or of proteins containing an equivalent of that amino acid. Further confirmation would seem to be available from the fact that by increasing the casein from 5 per cent to 20 per cent so that the total amounts of proteins reached 29 per cent (Diet 15), the fatty liver was prevented.

When, however, maize and 5 per cent dried skimmed milk were fed to rats (yielding an overall protein content of 9.1 per cent), the liver did not show any fatty changes (TABLE 1 Diet 4). Similarly, when a diet containing glucose and 20 per cent skimmed milk powder (TABLE 1 Diet 14) yielding a total of 6.20 per cent protein was fed to young rats, the fatty liver was again prevented. It might be argued that 6.2 per cent of dried skimmed milk protein provided sufficient methionine to prevent the fatty liver. On the other hand, 5 per cent dried skimmed milk in the maize diet (Diet 16) contains about one-third the protein in 5 per cent casein. The latter diet favored the accumulation of fat and the former did not. Five per cent casein contains more methionine than does 5 per cent dried skimmed milk. Yet the dried skimmed milk is more effective in preventing the fatty liver induced by a predominantly maize diet. It should not be forgotten, however, that, whereas casein is a pure protein, dried skimmed milk contains in addition to some proteins, other factors which constitute approximately 69 per cent of the dried skimmed milk. These other factors were apparently as important as the small amount of protein in preventing hepatic damage. It is imperative to stress the fact that dried skimmed milk or even whole milk cannot be regarded as consisting only of proteins; however efficient dried skimmed milk may be in treating malnourished infants in Uganda [Dean, 1952; Trowell *et al.* (1952), Editorial (British Medical Journal, 1952)]. Since we have already indicated that the omission of salts from the maize diet can prevent the fatty liver (compare Diets 9 and 10) it follows that explanations of the complex chemical processes underlying the production of the fatty liver in terms of single factors are untenable.

From these seemingly paradoxical observations it is evident that as in

The
setting

constellation promoting hepatic steatosis (compare Diets 10 and 11 with Diets

pected in nutritional investigations. Thus, the addition of 10 per cent food yeast to potato starch results in 95 per cent incidence of hepatic necrosis in young rats, whereas 10 per cent food yeast and 90 per cent maize meal is invariably associated with severe deformity of the bones. Obviously, the food yeast-maize combination provokes the pathological changes in the rats by virtue

By devoting exclusive attention to the deficiencies of maize, the peculiar properties of the constituents of maize are quite naturally overlooked. It is common knowledge that zein, the main protein of maize, contains hardly any lysine and practically no tryptophane. Proteins which contain considerable

published information, the unbalanced amino acid content of maize is less well appreciated. Six amino acids namely glutamic acid, tyrosine, phenylalanine, proline, leucine, and isoleucine account for 78 per cent of the total amino acids in zein. In this connection, too, it might be added that 62 per cent of the amino acids of gliadin of wheat are made up of glutamic acid, proline, leucine, and isoleucine. This disproportionate concentration of some amino acids can have serious consequences for the organism and, undoubtedly, can acquire toxic properties in some dietary settings. Several investigators have disclosed that, in appropriate circumstances, feeding excessive amounts of one amino acid can excite severe toxic reactions in experimental animals. One need only refer to the acute necrosis of the pancreas following the exhibition of excess tyrosine (Hueper and Martin, 1943). However, even in physiological concentrations, an amino acid such as cystine may be required in some dietary settings to produce hemorrhagic nephritis or acute necrosis of the liver (Griffiths and Wade, 1939). Moreover, it has been shown that an excess of one amino acid, such as glycine, calls for an increase in the pteroylglutamic acid in the diet if the growth-inhibiting effect of glycine is to be prevented (Dinning *et al.*, 1949).

That is to say, an abnormally high concentration of an amino acid sharpens the need for particular vitamins and other food factors in amounts which are out of proportion to the requirements of the organism subsisting on a different diet.

determine whether or not fat will accumulate in the livers of animals. The numerous reports published during the last decade afford support for this belief. Without wishing to reopen the entire problem of relation of proteins to the fatty liver, we should like to mention at this stage that our own investigations reveal the unsettled role of proteins in or preventing the fatty liver.

From TABLE 1, it is evident that the fatty liver develops in young rats on Diet 7 and Diet 11. In heavy weight (adult) rats, maize alone is efficient in fatty liver producing diet. If we examine the protein content of all these diets (TABLE 1), it is clear that in our experiments the fatty liver was produced at protein concentrations ranging from 8.1 per cent to 15 per cent. In the case of the maize alone (Diet 1), the fatty liver could be prevented or cured by administration of 50 mg of methionine per rat per day. This latter observation would seem to indicate that, even in diets with over 15 per cent protein there is still a lack of methionine or of proteins containing an equivalent of that amino acid. Further confirmation would seem to be available from the fact that, by increasing the casein from 5 per cent to 20 per cent so that total amounts of proteins reached 29 per cent (Diet 15), the fatty liver was prevented.

When, however, maize and 5 per cent dried skimmed milk were fed to (yielding an overall protein content of 9.1 per cent), the liver did not show fatty changes (TABLE 1, Diet 4). Similarly, when a diet containing glucose and 20 per cent skimmed milk powder (TABLE 1, Diet 14) yielding a total of 6.20 per cent protein was fed to young rats, the fatty liver was again prevented. It might be argued that 6.2 per cent of dried skimmed milk protein provided sufficient methionine to prevent the fatty liver. On the other hand, 5 per cent dried skimmed milk in the maize diet (Diet 16) contains about one-third the protein in 5 per cent casein. The latter diet favored the accumulation of fat and the former did not. Five per cent casein contains more methionine than does 5 per cent dried skimmed milk. Yet the dried skimmed milk is not effective in preventing the fatty liver induced by a predominantly maize diet. It should not be forgotten, however, that, whereas casein is a pure protein, dried skimmed milk contains in addition to some proteins other factors which constitute approximately 69 per cent of the dried skimmed milk. These other factors were apparently as important as the small amount of protein in preventing hepatic damage. It is imperative to stress the fact that dried skimmed milk or even whole milk cannot be regarded as consisting only of proteins, however efficient dried skimmed milk may be in treating malnourished infants in Uganda [Dean, 1952; Trowell *et al.* (1952), Editorial (*British Medical Journal*, 1952)]. Since we have already indicated that the omission of salts in the maize diet can prevent the fatty liver (compare Diets 9 and 10), it follows that explanations of the complex chemical processes underlying the production of the fatty liver in terms of single factors are untenable.

From these seemingly paradoxical observations it is evident that as a case of the production of hepatic necrosis a minimum number of nutrients must be present in the diet to promote the accumulation of fat in the liver. The nature of the metabolism stimulated by these nutrients provides the setting

In searching for proteins to supplement maize it is not only desirable to find protein containing sufficient amounts of lysine and tryptophane but care should also be taken not to increase still further the already high level of glutamic acid known to be present in maize. Our experimental studies have led us to assert repeatedly that, although a minimum number of food factors is required by the organism to promote growth and differentiation, the precise requirements of the organism for any particular food factor are a function of the quality and quantity of the other constituents of the diet.

In the present state of knowledge, we may affirm that generalizations based on particular experiments about the requirements of organisms for particular food factors are not only unjustified but can be grossly misleading in the management of nutritional disease (Gillman and Gillman 1951). Such generalizations are all the more to be condemned when they are founded on short term experiments or merely on the growth curves and survival of the animals.

Equally unjustified are the assertions of Brock and Autret (1952) that dried skimmed milk is synonymous with protein. As mentioned above approximately 30 per cent of dried skimmed milk consists of protein. We ourselves have demonstrated that, whereas dried skimmed milk may assist in the recovery of pellagrous infants, thus confirming the original observations of Waterlow (1948), the addition of an equivalent amount of casein had no or beneficial effect.

The constellation of factors governing the integrity of the liver and of other tissues is now so great that any claim made for the efficiency of a particular factor in safeguarding the liver and other tissues can have only a limited application. In approaching the problem of human malnutrition it is as well to remember the timely observations of Adolph (1943), made in connection with water metabolism: "No sign from heaven is likely to point out one that unlocks the elementary activities. The theory that a single governor exists for a physiological activity does not appear now to be substantiated. Moreover, there is no means of recognizing a regulator even if examined. A specific volley of nerve impulses or an isolatable extract are possible links in the chain or elements in a complex. Repeatedly it is found that many factors vary simultaneously, each one is as central as any other and only by convenience of thought is one exalted above another."

References

- ADOLPH E F. 1943. Physiological Regulations. Jacques Cattell Press.
 ALTMANN A. 1948. Clin Proc (Cape Town) 7: 32.
 BABLET J & L NORMET. 1937. Bull acad m d (Paris) 117: 242.
 BROCK J F & M AUTRET. 1952. Kwashiorkor in Africa. World Health Organization Monograph Series (8). Geneva.
 CAMERON G R & W E A KARUNARATNE. 1936. J Path Bact 42: 1.
 CHAIKOFF I L, C L CONNOR & G R BISHOP. 1938. Am J Path 14: 101.
 CHAIKOFF I L, T GILLMAN, C ENTENMAN, I F RINEHART, & F L REICHERT. 1948. Exptl Med 88: 373.
 DEAN R F A. 1952. Brit Med J 791.
 DIBLE J H. 1951. Brit Med J 833.
 DIBLE J H, J MCMICHAEL, & S P V SHERLOCK. 1943. Lancet 2: 407.
 DENNING J S, C K KEITH, P L DAY & J R TROTTER. 1949. Proc Soc. Exptl Med 72: 267.

being must be accepted. When we use the word "activity" in this paper, we mean activity *in vitro* under stated conditions.

In theory, there are several ways in which enzyme activity or concentration might be affected by dietary deficiency. Since enzymes are proteins and, therefore, presumably in a state of rapid turnover, the rate of renewal of the enzyme molecule may be reduced if the supply of dietary protein is inadequate. There is indeed, ample experimental evidence that deficiency of protein or of single amino acids can cause changes in the enzymatic pattern of the cell, some enzymes being reduced, other selectively preserved^{8,11}. Although no clear picture with physiological meaning has yet emerged from this work, it is reasonable to suppose that in man as in animals dietary deficiency may cause qualitative changes, if not actual disruption, in the enzymatic machinery of the cell.

Another possible effect of malnutrition on enzymes is a kind of disuse atrophy. Potter,¹² discussing the purpose and justification of enzyme assays, wrote "The determination of the amount of enzyme in a cell would have no value whatever, were it not for the fact that the amount of an enzyme is probably closely related to the extent of its use". He was considering the enzyme pattern in relation to cell growth and specialized function. It is only a short step from this idea to the possibility of a loss of enzyme from lack of substrate, the converse of the adaptive enzyme formation that is known to occur in bacteria, and of which there seem to be some examples in mammalian tissues^{1, 13}. If such a process does occur, the effects of protein deficiency should be most apparent in those tissues which are most active in the formation or metabolism of protein. It is perhaps, more than a coincidence that in kwashiorkor where protein deficiency plays a prominent part the clinical and pathological evidence shows that the organs most affected are those which are known on physiological grounds and from isotope work to have the highest protein turnover namely pancreas, liver and gut. It is clear that if the synthetic mechanisms are damaged, either from lack of components or from disuse, the condition must become progressively more difficult to reverse. This may explain why such a long time is sometimes needed to restore the malnourished body to normal. Not only do the building blocks have to be supplied, but the synthetic machinery must itself be rebuilt.

Finally enzyme activity may also be affected by shortage of essential co-factors^{14, 15}. This point needs no elaboration. In this connection however, the work of Jacquot¹⁶ re-emphasizes the difficulty of making deductions about the behavior of the whole animal from the results of experiments *in vitro*. He has shown that, even in fatal pyridoxin or biotin deficiency there is no change in over all nitrogen metabolism or in the output of urea in the urine.

These are some of the speculations underlying this work. It is clear that even if changes in enzyme activity are found their interpretation will not be easy.

Material and Methods

All this work has been done on infants between the ages of six months and two years. The plan of investigation was simple. As soon as possible after

ENZYME ACTIVITY IN FATTY LIVERS IN HUMAN INFANTS

By J C Waterlow and S J Patnick

Medical Research Council, London, England, and Department of Physiology, University College of the West Indies, B W I

This paper is an account of work in progress, presented in the hope that it may give some clue to fruitful lines of attack in the future. Moreover, since the work is in a provisional stage, we shall perhaps be forgiven if more attention than usual is given to objectives and methods, rather than to actual results.

Our studies on the activity of enzymes in the human liver are based on the very simple idea that if, in man as in animals, structural damage to the liver can be caused by dietary means, that damage should be preceded by a metabolic change or biochemical lesion. If a proper perspective is to be kept, however

It may, therefore, be legitimate to regard liver injury not as a local phenomenon but as a particular example of the effect of an adverse environment on glandular organs throughout the body. This aspect has been very little studied in the rat. Secondly, in the case of the liver, although the broad similarities are suggestive, there is not an exact correspondence between the types of injury produced experimentally in the rat and those found clinically in man. Fat liver in man differs from its analogue in the rat both in pathology and in respect to treatment. In man, the fat is peripheral,¹ in the rat, central.² This difference is surely not without significance. In man, a high protein diet and particularly milk, seems to be more effective in removing fat than either choline or methionine.³ Again although human diets in many parts of the world are low in sulphur amino acids, there is no good evidence that massive necrosis of purely dietary origin occurs in man. On the other hand, some types of liver damage in association with malnutrition or a low protein intake, which as yet there is no experimental analogue, have been described in man. These include pigment cirrhosis in South Africa,⁴ infantile cirrhosis in India and serous hepatitis in Jamaica.⁵ For these reasons we must be cautious in applying to man the results of animal experiments, a conclusion that is supported by some of the biochemical data reported in this paper.

Few people however would now deny that there is such a thing as nutritional liver injury in man. In the search for what the Gilmans⁷ have called the "altered metabolic regulations" underlying the structural changes, it seemed best to concentrate on the dynamic aspects rather than to measure the levels or concentrations of metabolites in the tissue. The study of enzyme activity promised to be a useful tool, more sensitive than the classical liver function tests and going nearer to the root of the problem. There is well be, of course, a great difference between the activity of an enzyme measured *in vitro* under standardized and artificial conditions and the activity in the natural environment in the body. In the former case, we are measuring what it can be made to do, not what it actually does. This limitation for the

being must be accepted. When we use the word "activity" in this paper, we mean activity *in vitro* under stated conditions

single amino acids can cause changes in the enzymatic pattern of the cell, some enzymes being reduced, other selectively preserved.^{8,12} Although no clear picture with physiological meaning has yet emerged from this work, it is reasonable to suppose that, in man as in animals, dietary deficiency may cause qualitative changes, if not actual disruption, in the enzymatic machinery of the cell

Another possible effect of malnutrition on enzymes is a kind of disuse atrophy. Potter¹⁴ discussing the purpose and justification of enzyme assays wrote "The determination of the amount of enzyme in a cell would have no value whatever, were it not for the fact that the amount of an enzyme is probably closely related to the extent of its use". He was considering the enzyme pattern in relation to cell growth and specialized function. It is only a short step from this idea to the possibility of a loss of enzyme from lack of substrate, the converse of the adaptive enzyme formation that is known to occur in bacteria, and of which there seem to be some examples in mammalian tissues.¹⁵ If such a process does occur, the effects of protein deficiency should be most apparent in those tissues which are most active in the formation or metabolism of protein. It is perhaps, more than a coincidence that in kwashiorkor, where protein deficiency plays a prominent part the clinical and pathological evidence shows that the organs most affected are those which are known on physiological grounds and from isotope work to have the highest protein turnover, namely pancreas liver and gut. It is clear that if the synthetic mechanisms are damaged, either from lack of components or from disuse, the condition must become progressively more difficult to reverse. This may explain why such a long time is sometimes needed to restore the malnourished body to normal. Not only do the building blocks have to be supplied but the synthetic machinery must itself be rebuilt.

Finally enzyme activity may also be affected by shortage of essential co-factors.^{16,17} This point needs no elaboration. In this connection however, the work of Jacquot¹⁸ re-emphasizes the difficulty of making deductions about the behavior of the whole animal from the results of experiments *in vitro*. He has shown that even in fatal pyridoxin or biotin deficiency there is no change in over all nitrogen metabolism or in the output of urea in the urine.

These are some of the speculations underlying this work. It is clear that even if changes in enzyme activity are found, their interpretation will not be easy.

Material and Methods

All this work has been done on infants between the ages of six months and two years. The plan of investigation was simple. As soon as possible after

ENZYME ACTIVITY IN FATTY LIVERS IN HUMAN INFAN

By J C Waterlow and S J Patrick

Medical Research Council, London, England, and Department of Physiology, University of the West Indies, B W I

This paper is an account of work in progress, presented in the hope it may give some clue to fruitful lines of attack in the future. Moreover the work is in a provisional stage, we shall perhaps be forgiven if more attention than usual is given to objectives and methods, rather than to actual results.

Our studies on the activity of enzymes in the human liver are based on a very simple idea that if, in man as in animals, structural damage to the

rat. Secondly, in the case of the liver, although the broad similarity is suggestive, there is not an exact correspondence between the types of damage produced experimentally in the rat and those found clinically in man. The liver in man differs from its analogue in the rat both in pathology and in response to treatment. In man, the fat is peripheral,¹ in the rat, central.² The difference is surely not without significance. In man, a high protein diet, particularly milk, seems to be more effective in removing fat than either choline or methionine.³ Again although human diets in many parts of the world are low in sulphur amino acids there is no good evidence that massive liver damage of purely dietary origin occurs in man. On the other hand, some types of liver damage in association with malnutrition or a low protein intake, which as yet there is no experimental analogue, have been described in man. These include pigment cirrhosis in South Africa,⁴ infantile cirrhosis in India,⁵ and serous hepatitis in Jamaica.⁶ For these reasons we must be cautious in applying to man the results of animal experiments, a conclusion that is supported by some of the biochemical data reported in this paper.

Few people however would now deny that there is such a thing as functional liver injury in man. In the search for what the Gilmans⁷ have termed the "altered metabolic regulations" underlying the structural changes, we seemed best to concentrate on the dynamic aspects, rather than to try to measure the levels or concentrations of metabolites in the tissue. The study of enzyme activity promised to be a useful tool more sensitive than the traditional liver function tests and going nearer to the root of the problem. There will be, of course a great difference between the activity of an enzyme measured *in vitro* under standardized and artificial conditions, and the activity in its natural environment in the body. In the former case, we are measuring what it can be made to do, not what it actually does. This limitation for the

so that clearly our sample may not be representative. We have not felt it justifiable to try and get more than one specimen at any given time. The best, therefore, that can be done to solve this difficulty is to take two separate fragments from the same specimen (when enough tissue is available) and make

from one lobule to another rather than from one large area of the liver to another. In general, such comparisons have shown good agreement between duplicate homogenates.²²

The second difficulty is the basis of representation. Enzyme activity is usually expressed per milligram dry weight of tissue. In our material, dry weight must clearly be corrected for fat, and probably for glycogen also, but even when this is done, a true picture may not be obtained. As Kosterlitz²³ and others have shown, in malnutrition, the liver cell loses a large proportion of its cytoplasm, although the size of the nucleus remains unchanged. A diminution in enzyme activity per cell (which, presumably, is what matters) might, therefore, be masked by an increase in number of cells per unit weight. At first sight, the solution seems to be to use desoxynucleic acid as a standard of reference. In our material, however, this is open to two objections. In the first place, it is well known that there is a high degree of polyploidy in the liver. Although this is normally less marked in infants than in adults,²⁴ in our cases,

relatively true for the rat, but it is very far from true in man. Even in apparently normal livers, as many as one third of the cells are nonepithelial cells, and this proportion may be increased in the absence of any gross pathological lesion. It is common in our cases to find infiltrations of lymphocytes in livers that are otherwise histologically normal.

For these reasons although we have measured the DNA content of the liver in a number of cases, we cannot regard it as a satisfactory standard of reference. The best basis is probably protein nitrogen, provided there is no significant dilution with inert protein, such as collagen. Nitrogen figures are not yet available for the cases reported in this paper, and therefore the figures are expressed on the basis of fat free dry weight without correction for glycogen.

Results

Of the vast number of possible enzymes, only a few have so far been investigated. These have been chosen on two criteria: their physiological importance, and their concentration or activity. We expect to detect changes in this is approaching the reason that we have particularly sensi

the baby's admission to the hospital, biopsy of the liver was done by the transpleural route. The baby was then placed on a high protein diet, consisting mainly of milk, and biopsy was repeated after some four weeks. During this time, most cases showed a marked improvement in general condition, a gain in weight, although a few were refractory and improved much slowly.

During the past three years we have investigated cases of several different types. In some babies the clinical picture is dominated by severe weight loss, with no specific signs, and with a liver which, histologically, shows little deviation from normal. In others there is a fatty liver. This is usually accompanied by edema, with low concentrations in the plasma of total protein, albumin, and choline esterase. These cases may well be regarded as examples of kwashiorkor, although the characteristic lesions of skin and mucosae seem to be less common in Jamaica than in some parts of Africa. Yet other cases conform to the clinical and pathological picture of xerous hepatosis as described by Hill.⁶ In these, there is well marked hepatocellular degeneration in the liver. In general, however, we have avoided cases with evidence of advanced liver damage, as the interpretation of the findings is likely to be extremely difficult, and our main interest is in the detection of early changes.

In the present paper we have confined ourselves to cases with fatty livers. Even in such a restricted group, however, no two patients are exactly alike. Until much more material has been accumulated, it is not possible to treat them as a group, to average the results, and to evaluate them statistically in the laboratory. We have thought it best, therefore, simply to describe a few representative cases which illustrate the results obtained so far in spite of the danger of bias inherent in such a method. A further difficulty is that of obtaining control material from completely normal children. The best that we have been able to do is to use each case as its own control, comparing the results before and after treatment.

Details of the methods of handling the tissue and of the techniques used for the enzyme assays are given elsewhere.⁷ Experiments have been done by us and Holter.²⁰ This, in combination with the fact that it is possible to do duplicate assays of six or seven different enzymes together with estimations of fat, protein, and nucleic acids, on some 3 mg. (by weight) of liver. This amount of tissue is large compared with that used by some workers.²¹ For the present problem, however, there is no advantage in further scaling down the amount of liver used. On the other hand, although more than 3 mg. is often available, it is unwise to count on more than this in infants; it is not possible to insert the biopsy needle as deeply as in adults, and the pieces of tissue obtained are small. We also regard it as essential, at least half, and if possible more, should be put aside for histological examination.

Before going on to the results it is necessary to consider two technical points of some importance. The first is the question of sampling error. The 3 mg. of tissue represents only about 1/150,000 of the weight of the whole liver.

so that clearly our sample may not be representative. We have not felt it justifiable to try and get more than one specimen at any given time. The best, therefore, that can be done to solve this difficulty is to take two separate fragments from the same specimen (when enough tissue is available) and make two homogenates. Admittedly such fragments come from points in the liver only a few millimeters apart, but at least they represent different lobules. The histological findings suggest that the pathological processes with which we are dealing are essentially diffuse. Although there is some variation, it is from one lobule to another rather than from one large area of the liver to another. In general, such comparisons have shown good agreement between duplicate homogenates.²²

The second difficulty is the basis of representation. Enzyme activity is usually expressed per milligram dry weight of tissue. In our material, dry weight must clearly be corrected for fat, and probably for glycogen also, but even when this is done, a true picture may not be obtained. As Kosterlitz²³ and others have shown in malnutrition, the liver cell loses a large proportion of its cytoplasm, although the size of the nucleus remains unchanged. A diminution in enzyme activity per cell (which presumably is what matters) might, therefore, be masked by an increase in number of cells per unit weight. At first sight, the solution seems to be to use desoxynucleic acid as a standard

apparently as a response to injury. This fact destroys the basic postulate, that the DNA content per cell is constant. Secondly it is generally assumed implicitly by biochemists that the liver is a homogeneous tissue. This may be relatively true for the rat but it is very far from true in man. Even in ap-

in a number of cases we cannot regard it as a satisfactory standard of reference. The best basis is probably protein nitrogen, provided there is no significant dilution with inert protein such as collagen. Nitrogen figures are not yet available for the cases reported in this paper and therefore the figures are expressed on the basis of fat free dry weight without correction for glycogen.

Results

Of the vast number of possible enzymes only a few have so far been investigated. These have been chosen on two criteria: their physiological importance and their concentration or activity. We cannot expect to detect changes in activity in enzymes with a QO_2 of less than 1 because this is approaching the lower limit of sensitivity of our methods. It is for this reason that we have not studied xanthine oxidase, an enzyme which seems to be particularly sensi-

TABLE 1
COMPARISON OF THE ACTIVITY OF SOME LIVER ENZYMES IN MAN AND RAT

	Man*	Rat
	12	100
	4	50
	23	70
malic, QO_2	55	160
malic, QO_2	207	1000
glutamic, QO_2	0	-
Transaminase QCO_2	278	50
Choline esterase, QCO_2	8	9†

* The figures in this column are the average of results obtained in recovered cases

† Female rats

tive to adverse dietary conditions,^{11 12} because even in the rat its activity is low and in man, so far, we have not been able, in preliminary trials, to measure it with any accuracy.

Since there is almost no information in the literature, the first need is to get some idea of the pattern of enzyme activity in the normal human liver. As has been said, no strictly normal control series is available and therefore we use as a temporary standard of comparison, or yardstick, the average of all the figures obtained in recovered human cases. The results for eight enzymes, compared with those found by the same methods in the rat, are shown in TABLE 1. The difference in activity of the oxidative enzymes on the one hand, and of transaminase on the other, is too great to be explained by chance variations of sampling or by errors of technique. There seems to be a real difference in enzyme pattern between the two species, a difference which raises problems of some interest. It is clearly unsound to consider each enzyme in isolation. The presence of one in high concentration must affect the activity of others. The low level of succinoxidase must, it would seem, be a limiting factor in the activity of the Krebs cycle. Transaminases can interfere with this cycle by removing or adding substrates at three stages: pyruvate, oxalacetate, and α keto glutarate. These substances are, as Braunstein put it²⁵ at the metabolic crossroads, and may be important links through which the activity of one enzyme system can control or affect the rate of another. At present, we do not know very much about these interactions, but the great difference that has been found between rat and man is at least a stimulus to further investigation. It suggests that the normal metabolic paths may, perhaps not be the same in the two species, and supports the point of view already put forward, that we must not expect the effects of a given type of malnutrition to be identical.

These considerations form the background to our problem. We must now turn to the effect on activity or concentration of liver enzymes of malnutrition in general and of that type, in particular, which causes fatty infiltration. I do this, we can draw on the experience of some 30 cases investigated in the past but shall present, in detail, only the results obtained in three babies with severely fatty livers that have been studied recently (TABLES 2, 3, and 4).

TABLE 2
CASE 1 F AGE 6/12 WEIGHT 5.6 KG

	1st b opsy 9.1.53	2nd b opsy 3.11.53	Average*
Liver fat			
per cent of fresh weight	30.6	6.2	3
fat/nonfat solids $\times 100$	25.5	30	15
Plasma protein gm per 100 ml	3.7	5.7	6.5
Plasma choline esterase Michel units	0.35	11.56	0.6
Succinoxidase QO_2	9.7	4.2	4
Cytochrome-c reductase QO_2	18.5	25.9	23
Dehydrogenases			
lactic QO_2	59	58	55
malic QO_2	320	193	207
glutamic QO_2	10.0	9.4	9
Transaminase QCO_2	264	265	278

* The figures in the 3 columns are the average of results obtained in recovered cases

TABLE 3
CASE 2 F AGE 11/12 WEIGHT 6.6 KG

	1st b opsy 14.1.53	2nd b opsy 3.11.53	Average
Liver fat			
per cent of fresh weight	44.7	14.1	3
fat/nonfat solids $\times 100$	32.2	86	15
Plasma protein gm per 100 ml	4.1	6.0	6.5
Plasma choline esterase Michel units	0.50	0.70	0.6
Succinoxidase QO_2	5.1	5.5	4
Cytochrome-c reductase, QO_2	17.1	30.2	23
Dehydrogenases			
lactic QO_2	24.3	75.0	55
malic QO_2	176	119	207
glutamic QO_2	6.1	18.1	9
Transaminase QCO_2	210	307	278

TABLE 4
CASE 3 M AGE 11/12 WEIGHT 3.5 KG

	1st b opsy 9.1.53	2nd b opsy 20.1.53	Average
Liver fat			
per cent of fresh weight	39.5	3	3
fat/nonfat solids $\times 100$	33.5	19	15
Plasma protein gm per 100 ml	4.5	6.2	6.5
Plasma choline esterase Michel units	0.15	0.61	0.6
Succinoxidase QO_2	0.6	5.2	4
Cytochrome-c reductase QO_2	—	25.2	23
Dehydrogenases			
lactic QO_2	11.3	21.2	55
malic QO_2	185	133	207
glutamic QO_2	10.5	11.7	9
Transaminase QCO_2	425	326	278

From these tables, it can be seen at a glance that, in so far as we are looking for a consistent change in enzyme activity in the fatty liver, the results are negative. Nevertheless, it is worth while to consider each case very briefly in turn, because although all of them had a fatty liver, they showed marked clinical differences in other respects. We shall then try to see what indications the data provide for further lines of attack.

The first case (TABLE 2) was a girl aged 6 months, moderately underweight for her age. She had edema of the feet, hands, and back, with hypoproteinemia. The liver was enlarged and fatty, but there were no other signs of malnutrition. Her skin hair and mucosae were absolutely normal. Superficially, indeed this child appeared well nourished, because she had an ample cover of subcutaneous fat particularly on the face and limbs. She was, in fact, a perfect example of what has been called the "sugar baby." On treatment with skimmed milk powder, edema rapidly disappeared and the plasma protein concentration rose. The liver diminished in size and lost its fat. Weight gain however was slow. We formed the impression that body fat was being lost while muscle was being laid down. Whether or not this was the explanation, there was a great improvement in general condition in spite of the slowness of weight gain.

The second case (TABLE 3) was more severely underweight, but still retained a fair amount of subcutaneous fat. She had generalized edema, and the liver was enlarged and fatty. The striking difference between this child and first was the presence of very severe lesions of skin and mucosae—glossular stomatitis and desquamative dermatosis of the legs, arms and face. She was a typical case of kwashiorkor, as it is seen in Africa except that there was no dyspigmentation of the hair, no vomiting, and little diarrhea. There was a rapid response to treatment with skimmed milk, edema disappeared and thereafter there was a steady gain in weight. The liver was reduced in size and the skin lesions healed in a few days with no treatment other than milk.

Case 3 differed from the other two in being very severely underweight. Clinically, there was almost complete loss of both subcutaneous fat and of muscle and the child seemed to consist of little more than skin and bone. The skin showed a dry scaly dermatosis, but without the desquamation leaving an underlying raw red surface which was so prominent in Case 2. The hair in parts was quite white like that of an old man. In spite of the low plasma protein concentration there was no edema. The liver was only slightly enlarged but it was extremely fatty. A finding which, in our experience, is unusual in total starvation or marasmus. The response to treatment was very slow and it was only after three months in hospital that a steady weight gain began. Even before this however there was a gradual but undoubted improvement in that indefinable entity the general condition. Nitrogen balance measurements during this period showed a positive nitrogen retention, suggesting that body tissue was being built up gradually, presumably with a concurrent loss of water.

All these three cases had a great increase in liver f

usual method of

expressing fat as a percentage of fat, F, is calculated as $\frac{F}{S} \times 100$. If S = nonfat solids and W = wet weight, then $\frac{F}{W} \times 100$ seems more illuminating to give the ratio F/S, and this we have done in the second line of the tables, taking S as 100. On this basis, the fat content in Case 3 was some 20 times normal, whereas expressed as a percentage of the wet weight it was only 13 times normal.

This degree of fatty infiltration represents a gross perversion of metabolism which is not at all reflected in the figures for enzyme activity. There was a reduction in the plasma concentration of nonspecific or pseudo choline esterase. It has been shown in earlier work^{26, 27} that, in such cases, the liver esterase is also reduced, roughly *pari passu* with that in the plasma. Low levels of liver and plasma esterase are very constantly found in malnourished infants, but this does not seem to be related in any way to the presence or absence of a fatty liver. Rather it seems to be associated with severe weight loss and general malnutrition. It is also found, as is well known, when there is advanced structural damage to the liver, as in cirrhosis.

The dehydrogenases show rather variable results. In the first biopsy, the activity of the lactic enzyme was low in two of the three cases shown. This, however, is not a constant finding. In three other cases with fatty liver, the initial levels were not low. Malic dehydrogenase on the other hand, was present in higher concentration in the first than in the second specimen. This has been a rather consistent observation, not only in these, but in other cases also. The same is true for transaminase. In 11 out of 14 cases, transaminase activity was higher in the malnourished than in the treated liver. It would seem that both these enzymes are not only very active, but are well preserved, even selectively preserved. It would clearly be unwise in work of this kind, to confine our interest to enzymes which are reduced by dietary deficiency. The fact that some particular enzymes are jealously retained may equally be of significance. The figures for glutamic dehydrogenase activity show much scatter, and we have not yet been able to draw any conclusions from them.

So far, not very many measurements have been made of cytochrome reductase activity. It is too soon to attach any significance to the slightly low figures found in the initial biopsies of Cases 1 and 2. The results are included here in order to demonstrate one point only—that, in Case 2, there was a fair concentration of this flavoprotein enzyme in the liver in spite of the presence of severe signs which clinically are attributed to ariboflavinosis.

In the case of succinoxidase, the results again are variable. A very low activity, however, like that found in Case 3 has been observed in some other babies who were severely malnourished with or without fatty liver. We can not feel the same confidence about these results as in the case of the other enzymes, because on two occasions there has not been satisfactory agreement between duplicate homogenates. To admit the possibility of technical error or artifact need not however be entirely a confession of failure. Artifacts can be significant. The instability of the Krebs cycle enzymes probably arises from the fact that they are parts of an organized structure—parts which cannot

work if their interrelationship is disturbed. Succinoxidase, although less labile than the others, is a member of this group both functionally and structurally since it is found only in the mitochondria. In the assay of such enzymes since some degree of organization must be preserved, we are one step nearer to conditions *in vivo* than when measuring the activity of a soluble enzyme such as transaminase. It is conceivable that the effect of malnutrition (that is, of an adverse chemical environment *in vivo*) may be not to reduce the amounts of enzyme molecules but, in some way, to destroy their organization or to make it more easily destroyed under the conditions of the *in vitro* measurements.

particulates is not the same in the two species. Histologically, both mitochondria and basophil substance are much more sparse in the human liver than in that of the rat. This is confirmed by preliminary measurements that we have made of the ribonucleic acid content of the liver.²⁷ In our cases, this was very low. The ratio of RNA to DNA was about 1:1, compared with about 4:1 in the rat. Because of the doubt raised by Rossiter and his colleagues²⁸ about the specificity of the color methods, and because of the possibilities of artifact arising from working in a tropical climate without a cold room, we have postponed pursuing this investigation further until a critical examination of the methods has been completed. For this reason, we do not present here data on the nucleic acid content of the fatty liver. But the point has been raised in order to emphasize again the need for more basic information about the human liver.

In conclusion, in these fatty livers the activity of those enzymes which have been measured is, on the whole, well maintained. The only enzymes which have been found, in these and other malnourished cases, to be reduced in amount are the nonspecific choline esterase and perhaps succinoxidase. These changes seem, however, not to be related to the fatty liver *per se*, but to the general state of malnutrition of the infant. There may well be a connection between such a reduction in enzyme activity and the semi irreversible state which some of these infants seem to reach when malnutrition has been sufficiently severe or prolonged. The fatty liver is only one element in this picture, and not necessarily the most important. Although the term "fatty liver disease"²⁹ has its uses (it might be a more appropriate label for Case 1 than Kwashiorkor), it must not be used to imply, as one of us did five years ago,¹ that the fatty liver is the dominant feature on which a prognosis can be based. This position can no longer be held. When these babies die, in all likelihood it is not the fatty liver that kills them, but something else which, for want of a better term, we can only call generalized protein malnutrition. The severity of this does not necessarily run hand in hand with the degree of fatty infiltration in the liver.

Since there are many variables in this clinical picture, the collection of facts that can be classified, compared and analyzed, is a slow process. We must apologize for presenting, in the meantime, a piecemeal account of work that

has been done on such a small number of cases. Our justification is the hope that others will pursue this path, so that knowledge may be gained more quickly.

APPENDIX

Methods

The liver specimens are obtained with a Vim Silverman needle inserted by the transpleural route. The infant is given paraldehyde *per rectum* before hand and 2 per cent novocaine is used to infiltrate the skin at the point of incision. The tissue is transferred from the needle into ice-cold saline. A

there is enough tissue to make duplicate homogenates, and each piece is weighed on a quartz fibre torsion balance. The balance that we use was constructed in the laboratory according to the principle illustrated by Strong²². The only modification is that we do not fuse the fibre to the beam, which is extremely difficult but seal it with a special resin (Araldite, made by Aero Research Ltd., Duxford, Cambridge, England). This has proved very satisfactory. The zero of the balance has remained unchanged for 18 months. The only disadvantage of this method is that it is not possible to coat the balance with platinum by painting with chloroplatinic acid and flaming in order to prevent the accumulation of electrostatic charges. However, at the level of sensitivity at which we work, and in the rather humid climate of the tropics, we have not had any trouble from this source. We have not aimed at a sensitivity greater than 2 μ g. With 2 mg of tissue the error of weighing is still much less than the other errors of the chemical or enzymatic measurements.

One piece, after weighing, is put over phosphorus pentoxide to dry, and reweighed the next day, to give the water content. This piece should if possible weigh at least 1 mg, fresh weight, or $\frac{1}{4}$ mg dry weight, although the chemical measurements can be done with less. The dried liver is put into a micro Soxhlet apparatus similar to that described by Kirk²⁶ extracted with hot ether, dried and weighed again. This method of measuring fat content by difference after ether extraction gives results that are a little low. It is certain that some of the phospholipid is left behind. In the normal liver, this method gives a fat content of about 3 per cent of the wet weight, compared with the accepted normal value of 5 per cent. The difference is consistent, however, and is of little importance for our purpose since the neutral fat does seem to be taken out quantitatively.

In the past we have used this dried defatted tissue for measurements of nucleic acids and of protein nitrogen. The dry specimen was homogenized and extracted with hot trichloroacetic acid, according to the method of Schneider². The final volume of extract was about 30 cu mm. Total nucleic acids were measured in the extract by ultra violet absorption after diluting 25 times with water. In this way, the TCA blank becomes negligible. DNA was measured by the diphenylamine reaction. As this reaction is not very sensitive it was

necessary to have a final volume of not more than 20 cu mm., and this involved using a microscope colorimeter. RNA was measured by difference. After extraction with TCA, protein nitrogen was measured in the residue by digestion and nesslerization.

The other piece of tissue is used for the enzyme assays, and should weigh not less than 2 mg. We homogenize in a volume of 20 cu mm., and aim at a tissue concentration of about 10 per cent. If less than this is used the accuracy of measurement suffers for those enzyme systems, like succinoxidase, which have a low QO_2 . The tissue is homogenized in ice cold 0.88M sucrose in a tube 2 to 3 mm. in diameter. The pestle of the homogenizer is made of glass and is rotated by a motor. The conditions of homogenization are kept as standardized as possible. During homogenization, which lasts only for 30 seconds, the tube is held in a large brass block previously cooled on ice.

Once the homogenate has been made, it is necessary to work quickly. Of the enzymes investigated so far, we have not found any loss of activity in intact tissue that has been left on ice for several hours, but in a homogenate some enzymes do become inactivated at an appreciable rate on standing; notably succinoxidase. The dehydrogenases, transaminase and cholinesterase seem to be stable for many hours. Immediately after the homogenate has been made aliquots are removed and diluted for the assays of dehydrogenases and of cytochrome c reductase. For the former, the dilution is made in water, with a tissue concentration of about 5 μ g per cu mm. For the latter the dilution is made in 0.88M sucrose with a tissue concentration of about 1 μ g per cu mm. Two sets of measurements can then be done simultaneously: one by spectrophotometric, the other by gasometric methods. All the gasometric measurements have been made by the Cartesian diver technique of Linderström-Lang and Holter, as modified by Waterlow and Borrows,²² and by Borrows and Penney.²³ This technique is sensitive, accurate, and versatile, and the apparatus needs little time needed to fill the divers. To fill and measure more than 60 divers, the limitations of fatigue make it difficult for one person to do more than about 16 divers in one day.

Assay Methods

Gasometric. All assays have been done at least in duplicate. In the instance of succinoxidase the conditions chosen have been those which have been worked out for assays on rat liver. Whenever enough time and enough tissue have been available, measurements have also been made with varying concentrations of substrate and buffer etc. since it cannot be assumed that the optimum conditions are the same for man and the rat.

Succinoxidase. The method was that of Schneider and Potter.²⁴ **Diver filling (FIGURE 1).** Bottom drop 0.5 μ l of 2N NaOH. On the wall 0.25 μ l of a mixture containing cytochrome c (Wyeth) 2.5 mg per ml, $AlCl_3$ $3 \times 10^{-3}M$, $CaCl_2$ $2.5 \times 10^{-3}M$. Homogenate 1 μ l of sucrose homogenate, strength about 10 per cent. To this was added 0.25 μ l of a mixture

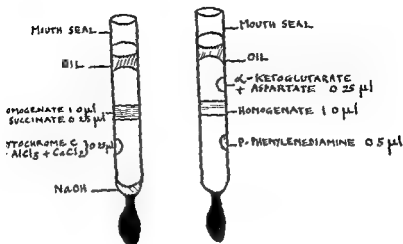


FIGURE 1 (left) Filling for succinate dehydrogenase assay FIGURE 2 (right) Filling for transaminase assay

drop containing cytochrome c . Readings were made for one hour. A blank was always run containing all reagents except succinate because at this high succinate concentration the endogenous respiration is appreciable ($QO_2 = 1$ to 2).

Transaminase. Our method is essentially that of Ames and Elvehjem,¹⁵ in which the reaction measured is the formation of glutamic and oxaloacetic acids from aspartic and α keto-glutaric acids. The oxaloacetate is then decomposed, and the CO_2 output measured. It was found however that activity was often not linear in relation to the amount of tissue being less with large amounts. The same effect was found when the time of incubation was varied, the activity being relatively less with incubations of 15 or 20 minutes than of 5 or 10 minutes. It is probable that this arises from the position of the equilibrium point in this reaction, which is reached when about $\frac{1}{3}$ of the aspartate and α keto-glutarate have reacted.¹⁶ The effect has been reduced but not eliminated by increasing the concentration of substrates. The strength of the homogenate has been kept as constant as possible and measurements have been made in duplicate with only one strength of tissue incubated for 5 and 10 minutes. For decomposing the oxaloacetate we have used a solution of p phenylenediamine instead of aniline citrate. With this, evolution of CO_2 is complete in about 5 minutes. Aniline citrate has the disadvantage of being oily, so that it cannot be placed as a discrete drop on the diver wall.

Diver filling (FIGURE 2). Lower drop 0.5 μ l of M/1 p-phenylenediamine in N/1 HCl. Homogenate 1 μ l of homogenate diluted to 2 per cent with 0.88M sucrose. Upper drop 0.25 μ l of a mixture containing aspartate 0.3M, α keto-glutarate 0.3M phosphate buffer 0.15 M pH 7.4. Homogenate and

upper drop mixed by applying a negative pressure after equilibration. Lower drop mixed by applying a positive pressure 5 or 10 minutes later. CO_2 output is complete in 5 to 10 minutes.

For the blank exactly the same filling is used, but the two mixings are carried out in immediate succession so that the incubation time is zero.

Choline esterase. *Diver filling.* 1.25 μl of homogenate. To this is added during the filling of the diver 0.25 μl of 0.19M NaHCO_3 and 0.3 μl of 25 per cent acetyl choline. *Gas-phase.* 5.2 per cent CO_2 in oxygen. Divers gas under water by the technique of Waterlow and Borrow.¹¹ Readings are taken from 15 to 45 minutes after the moment of adding the acetyl choline.

Spectrophotometric Methods

Dehydrogenases. The lactic, malic, and glutamic dehydrogenases were measured by following the reduction of DPN. Density measurements were made at 340 $\text{m}\mu$ with the Beckmann spectrophotometer. The details of the method were kindly supplied by Dr. J. S. Strominger of the National Institute of Health. The amounts of tissue used for the assays were of the order 5 μg for the lactic dehydrogenase, 2 μg for the malic dehydrogenase, and 50 μg for the glutamic dehydrogenase. The final volume was, in all cases, 0.110 ml. Results are expressed in terms of QO_2 . This calculated from the observed density changes by the use of Ohlmeier's figure of 6270 for the molecular extinction coefficient of DPNH_2 .¹⁷

DPN-Cytochrome-c Reductase. This enzyme was measured by a modification of the method of Brody *et al.*¹⁸ In this method the amount of reduced cytochrome c is determined by measuring the optical density at 520 $\text{m}\mu$. The amount of tissue used was of the order 0.110 ml. Results are expressed as QO_2 . This calculated from the observed density changes by the use of the molecular extinction coefficients of oxidized and reduced cytochrome c.¹⁹

References

1. DAVIES, J. N. P. 1948. *Lancet* 1: 317.
2. ASHBURN, L. L., K. M. ENDICOTT, E. S. DART, & R. D. LILLIE. 1947. *Am. J. Path.* 23: 159.
3. WATERLOW, J. C. 1948. Fatty Liver Disease in Infants in the British West Indies. Med. Research Council (Brit.) Special Rept. Series No. 263. H. M. Stationery Office, London.
4. GILLMAN, T. & J. GILLMAN. 1945. *Arch. Path.* 40: 239.
5. RAO, M. V. R. 1935. *Indian J. Med. Research* 23: 69.
6. HILL, K. R., K. RHODES, J. L. STAFFORD, & R. AUST. 1953. *Brit. Med. J.* 1: 117.
7. GILLMAN, J. & T. GILLMAN. 1951. *Perspectives in Human Malnutrition*. Grune & Stratton, New York.
8. MILLER, L. L. 1948. *J. Biol. Chem.* 172: 113.
9. SCHULTZ, J. 1949. *J. Biol. Chem.* 178: 451.
10. WILLIAMS, J. N. & C. A. ELVEHJEM. 1949. *J. Biol. Chem.* 181: 559.
11. ROSENTHAL, O., C. S. ROGERS, H. M. VARS, & C. C. FERGUSON. 1950. *J. Biol. Chem.* 185: 669.
12. WILLIAMS, J. N., A. E. DENTON, & C. A. ELVEHJEM. 1949. *Proc. Soc. Exptl. Biol. Med.* 72: 386.
13. BARGONI, N. 1951. *Experientia* 7: 104.
14. POTTER, V. R. 1950. In *Respiratory Enzymes*. Ed. H. A. Lardy. Burgess, Minneapolis, Minn.

- 15 KNOT, W E 1951 Brit J Exptl Path 32 462
- 16 MANDELSTAM, J & J YUDKIN 1952 Biochem J 51 681
- 17 AXELROD, A E & C A FLVERJEM 1941 J Biol Chem 140 725
- 18 SCHLENK, F & E E SNELL 1945 J Biol Chem 167 425
- 19 JACQOT R Personal communication
- 20 LINDERSTROM LANG K & H HOLTER 1943 Compt rend trav lab Carlsberg (Sér chim) 24 334, 399
- 21 STROMINGER J L & O H LOWRY 1952 Federation Proc 11 295
- 22 WATERLOW, J C 1952 Liver Injury Trans 11th Conf Josiah Macy, Jr, Found New York
- 23 KOSTERLITZ H W 1947 J Physiol 105 194
- 24 FRUHL, K 1932 In von Moellendorf's Handbuch der mikroskopischen Anatomie des Menschen Springer Berlin
- 25 BRAUNSTEIN, A E 1947 Advances in Protein Chem 3 1
- 26 WATERLOW, J C 1950 Lancet i 908
- 27 " " " " " " " "
- 28 " " " " " " " "
- 29 " " " " " " " "
- 30 " " " " " " " "
- 31 " " " " " " " "
- 32 " " " " " " " "
- 33 " " " " " " " "
- 34 " " " " " " " "
- 35 " " " " " " " "
- 36 " " " " " " " "
- 37 " " " " " " " "
- 38 " " " " " " " "
- 39 " " " " " " " "
- 40 " " " " " " " "
- 41 " " " " " " " "
- 42 " " " " " " " "
- 43 " " " " " " " "
- 44 " " " " " " " "
- 45 " " " " " " " "
- 46 " " " " " " " "
- 47 " " " " " " " "
- 48 " " " " " " " "
- 49 " " " " " " " "
- 50 " " " " " " " "
- 51 " " " " " " " "
- 52 " " " " " " " "
- 53 " " " " " " " "
- 54 " " " " " " " "
- 55 " " " " " " " "
- 56 " " " " " " " "
- 57 " " " " " " " "
- 58 " " " " " " " "
- 59 " " " " " " " "
- 60 " " " " " " " "
- 61 " " " " " " " "
- 62 " " " " " " " "
- 63 " " " " " " " "
- 64 " " " " " " " "
- 65 " " " " " " " "
- 66 " " " " " " " "
- 67 " " " " " " " "
- 68 " " " " " " " "
- 69 " " " " " " " "
- 70 " " " " " " " "
- 71 " " " " " " " "
- 72 " " " " " " " "
- 73 " " " " " " " "
- 74 " " " " " " " "
- 75 " " " " " " " "
- 76 " " " " " " " "
- 77 " " " " " " " "
- 78 " " " " " " " "
- 79 " " " " " " " "
- 80 " " " " " " " "
- 81 " " " " " " " "
- 82 " " " " " " " "
- 83 " " " " " " " "
- 84 " " " " " " " "
- 85 " " " " " " " "
- 86 " " " " " " " "
- 87 " " " " " " " "
- 88 " " " " " " " "
- 89 " " " " " " " "
- 90 " " " " " " " "
- 91 " " " " " " " "
- 92 " " " " " " " "
- 93 " " " " " " " "
- 94 " " " " " " " "
- 95 " " " " " " " "
- 96 " " " " " " " "
- 97 " " " " " " " "
- 98 " " " " " " " "
- 99 " " " " " " " "
- 100 " " " " " " " "

THE QUESTION OF THE RELATIVE IMPORTANCE OF PROTEIN AND LABILE METHYL IN THE DEVELOPMENT OF FATTY LIVER AND CIRRHOSIS IN MAN

By Bruce M. Nicol*

Colonial Medical Service Nigeria British West Africa

The hypothesis that fatty liver disease and cirrhosis in man is a cause of nutritional deficiency has never been proved,¹⁻¹⁰ but many clinicians working in temperate climates hold the view that the lesion develops in alcoholics when they cease to ingest a good mixed diet. Investigators working in the tropics believe that a low intake of protein is an important factor in the causation of "kwashiorkor" in children.¹¹⁻²³ Trowell²⁰ has stated that animal protein is particularly important in this respect.

Animal experiments have shown that at least part of the lipotropic effect of protein is the resultant of the opposing influences of cystine and methionine, that a deficiency of choline leads to fatty liver, and that hepatic fibrosis results from chronic interference with the utilization of methionine.² Other nutritional interrelationships are involved in the production of fatty liver in man. Cystine exerts a positive effect on the accumulation of liver fat only if there is low intake of niacin,²² and vitamin B12 and folic acid have been shown to influence the metabolism of choline and methionine.¹⁶ Some evidence suggests that unidentified factors may be present in the protein molecule which affect fat deposition.^{21, 24} A factor, as yet unidentified, has recently been purified from casein and from certain yeasts which protects rats against dietary protein liver degeneration.^{17, 18}

The present communication deals with the incidence of hepatomegaly, fatty liver and cirrhosis in three West African tribes and in one group of similar wealthy native traders as related to their diets and other environmental factors.

Methods and results A general description of the investigations carried out in Nigeria between 1947 and 1951, and the methods used to determine protein intakes of Nigerian natives, have been published elsewhere.^{25, 26} The samples of the tribes were formed of all the inhabitants estimated to be over 15 years of age in three small villages in the tribal areas. The traders lived in a town of some size in the center of the Niger Delta. Clinical enlargement of the liver was diagnosed if the organ was palpable two fingers below the costal margin in the midclavicular line, the subject lying on his back and breathing normally. Ascites and jaundice were encountered occasionally in association with hepatomegaly, gynecomastia and feminism were seen occasionally in males, spider naevi were noted in a few natives who had pale skins and emaciation was a prominent feature of the more advanced cases. Hepatomegaly was found at all ages but more frequently after the third decade. The lesion was observed more often in females than in males, but the differ-

* I am grateful to the Inspector General of Medical Services Nigeria for permission to publish this paper and to Doctor Klaus Schwarz, National Institutes of Health, U. S. Public Health Service, Bethesda, Md. for his helpful criticism.

as not statistically significant. Infective hepatitis is a common disease throughout Nigeria. Such cases of liver enlargement were excluded from this study.

Specimens of liver tissue were obtained by needle biopsy or by laparotomy.

in the sections.

The incidence of hepatomegaly in the four groups, together with the prevalent parasitic infestation rates, the calculated daily intake of alcohol, and the frequency of a past history of transient jaundice are shown in TABLE 1. The consumption of alcohol, a habit freely indulged by men, women, and often children, is highest in the traders, in which group the incidence of enlarged liver is least. The total parasitic load is approximately equal in the three tribes, and it is believed that nutritional demands made, and any hepatic damage caused, by parasitic toxins or the parasites themselves, are likely to be of the same degree in each tribe. Clinical disease and pathological states attributable to specific parasites are seen less often than would be expected from the numbers of natives who carry these organisms. The incidence of hepatomegaly is

TABLE 1

THE INCIDENCE OF HEPATOMEGALY, THE PREVALENT PARASITIC INFESTATION RATES, THE DAILY ALCOHOL CONSUMPTION, AND THE FREQUENCY OF A PAST HISTORY OF TRANSIENT JAUNDICE IN THREE WEST AFRICAN TRIBES AND IN ONE GROUP OF WEALTHY TRADERS

	Traders (90)*	Igbo (112)*	Igbo (121)*	Dahomeans (104)*
	%	%	%	%
Incidence of hepatomegaly	3	8	19	11
Parasites ‡				
Schistosomes	1	11	2	43
E. histolytica	4	8	3	3
Ankylostomes	73	96	95	65
Ascaris	17	20	51	5
G. lamblia	3	4	2	11
Dracontius	0	0	0	7
Filaria				
loa loa	4	4	3	11
bancrofti	0	0	11	3
Splenic index (children 2-10 years)	17	17	22	17
Previous history of transient jaundice	3	8	6	6
	ml.	ml.	ml.	ml.
Alcohol consumption—average daily intake of				
Home brewed beer (2-5%)	0	11	0	228
Palm wine (1-7%)	120	105	250	0
Local spirits (60-70%)	20	107	13	0
Imported beer (4-6%)	100	0	0	0
Imported spirits (50-70%)	63	11	0	0

* Numbers examined clinically.

† Figures in parentheses denote alcoholic content.

‡ Number of individuals found to be carriers of parasite or its ova, not all suffering from specific disease as a result of the infestation.

TABLE 2

CALCULATED DAILY INTAKE OF NUTRIENTS OF THREE NIGERIAN TRIBES AND OF ONE OF WEALTHY TRADERS

Group	Protein		Fat	ChO	Vitamin A*	Thiamine	Riboflavin	Niacin	Vt. C	Fe	Ca
	An	Veg									
	g	g	g	g	IU	mg	mg	mg	mg	mg	mg
Traders	50	34	86	473	9200	1.02	1.37	18.1	71	21	1302
Ijaws	68	12	39	379	5700	0.30	0.88	12.1	40	24	2800
Isokos	20	26	43	424	7400	0.69	0.65	8.1	30	21	664
Dakarkerris	2	85	24	595	700	3.70	0.98	25.0	34	57	581

	Contribution to calories (%)			Composition of diets by weight		
	Protein	Fat	Carbohydrate	Protein	Fat	Carb.
Traders	11.2	25.8	63.0	13.1	13.4	73.5
Ijaws	14.6	16.4	69.0	16.1	7.8	76.1
Isokos	8.1	17.1	74.8	9.0	8.4	82.6
Dakarkerris	11.8	7.3	80.9	12.3	3.4	84.3

* 1 I.U. of Vitamin A taken as equivalent to 3 I.U. of Vitamin A in the form of carotene.
No allowance made for destruction of nutrients by cooking.

least in the traders (3 per cent) and greatest in the Isoko (19 per cent), corresponding figures for the Ijaws and Dakarkerris being 8 per cent and 19 per cent respectively, the difference between the rate in the Isoko and the other two tribal groups being significant ($P = 0.01$). A correlation between hepatomegaly on the one hand and a previous history of jaundice or any other splanchnic infestation on the other hand was not found.

Full details of the foodstuffs eaten are given in the appendix. The calculated daily intake of nutrients is given in TABLE 2. It will be seen that the amounts of individual nutrients vary widely from group to group, resulting from the use of different staple foods in the different regions of Nigeria. The traders' diet is considered to be reasonably adequate, but the three tribes each show grave deficiencies of one or more nutrients. A low intake of protein, however, is the only feature by which the Isoko diet is distinguished from the diets of the other two tribes. The Isokos ingest 46 gm of protein daily, of which 20 gm is derived from animal sources, the Ijaws' diet from 68 gm, and the Dakarkerris' diet from 50 gm.

For the purpose of calculating the nitrogen requirements of man, numerous analyses of the amino acid content of foodstuffs, choosing an average according to species, soil, and climate, have been made. For these analytical data, I am indebted to Doctor M. Autret of the Food and Agricultural Organization. Sufficient data applicable to African foodstuffs has not been found from which to assess the cystine content of the diets. The results, together with the amounts of protein nitrogen ingested,⁴ are given in TABLE 3. Comparison is made between the essential amino acid composition of the Nigerian diets and the requirements of man advocated by Rose.¹⁰ TABLE 3 also includes an estimate of the nitrogen requirements of man as calculated.

TABLE 3

Nutrient	Rose (1949)		Traders	Ijaws	Isokos	Dakarkerns
	Maintenance*	Allowance				
Calories	3600	3600	3000	2200	2300	2900
Protein (g)						
Animal			50	68	20	2
Vegetable			34	12	26	85
Nitrogen† (g)	10	10	13	12	7	13
Essential amino acids (g)	6.4	12.8	30.3	28.8	15.8	27.2
Iso-leucine	0.7	1.4	4.1	3.8	2.0	3.6
Leucine	1.1	2.2	6.5	5.8	3.2	8.3
Lysine	0.8	1.6	5.3	5.8	2.7	2.1
Methionine	1.1	2.2	2.0	2.2	1.2	1.3
Phenylalanine	1.1	2.2	3.5	3.0	1.9	3.4
Threonine	0.5	1.0	3.4	3.3	1.8	3.1
Tryptophan	0.3	0.5	1.0	0.8	0.6	0.9
Valine	0.8	1.6	4.5	4.1	2.4	4.5
Choline (g)			0.92	0.65	0.60	0.65
Incidence of hepatomegaly (%)			3	8	19	9

* Minimal requirements for maintenance of nitrogen in healthy young American adult males

† Does not include any nonprotein nitrogen present in the foodstuffs used

the choline content of the African diets, which has been made from such published data as are available.^{6, 7, 8, 11} For some of the foods, the choline and amino acid contents had to be derived by analogy.

If Rose's daily recommendations are considered, the only deficiencies of essential amino acids detectable are those of methionine in the diets of the traders, Isokos and Dakarkerns and of phenylalanine in the Isoko diet. Of the eight essential amino acids, each group ingests more than Rose's minimal requirements for the maintenance of nitrogen balance in healthy adult Ameri-

reason does not exist to suppose that the Dakarkern diet contains considerably more cystine than that of the Isokos.

Discussion The data presented above show that the only feature whereby

Enlargement of the liver undoubtedly results from invasion of the organ by parasites as in amoebic hepatitis and schistosomiasis and part of the total incidence of hepatomegaly in all groups is due to such causes but these lesions

are manifested by focal necrosis and abscess formation, patchy fibrosis and associated regeneration of liver tissue, not by fatty infiltration and uniform cirrhosis. A large percentage of the sections of liver from individuals suffer from hepatomegaly in this series showed the latter picture. It has been pointed out above that the incidence of parasitic infestations does not parallel the incidence of hepatomegaly, and it is believed that nutritional factors account for the varying incidence of this clinical feature in the tribes under consideration.

In view of the above discussion, and as a low intake of protein is the one feature by which the Isoko can be distinguished from the other groups considered, a further argument is advanced to explain the high incidence of hepatomegaly in this tribe—namely, that some unidentified factor, which occurs in, or is associated with, protein is involved in the protection of the liver from fatty infiltration and cirrhosis, and possibly also from the effects of parasitic toxins and alcohol. The existence of such a factor has been suggested in the literature cited in the introduction to this paper. Such a factor would appear to be present and active both in vegetable and animal protein and therefore is unlikely to be related to vitamin B12.

Summary (1) The incidence of hepatomegaly (as defined) in three Nigerian tribes and in one group of wealthy native traders is recorded together with a description of the diets eaten, the histological appearance of the liver lesions, the prevalence of parasitic infestations, and the alcohol consumption of each group.

(2) The importance of dietary protein in the etiology of the disease is discussed. The evidence obtained does not support the view that lack of one of the essential amino acids or lack of labile methyl in the diet is of importance in the development of fatty liver and cirrhosis in the groups investigated.

(3) It is suggested that some unidentified factor, which occurs in association with both animal and vegetable protein, plays a part in preventing development of fatty liver and cirrhosis in man.

APPENDIX

AVERAGE DAILY FOOD CONSUMPTION OF NIGERIAN PEASANTS AND TRADERS (ADULTS)

Foodstuff	Scientific name	Traders	Ijaws	Isokos	Do
Wheat flour (70%)	<i>Triticum vulgare</i> Host Cultigen	70	—	—	—
Rice milled	<i>Oryza sativa</i> Linn	76	—	—	—
Rice home pounded	<i>Oryza sativa</i> Linn	—	—	—	—
Maize mature yellow	<i>Zea mays</i> Linn	30	29	15	14
Maize immature		10	—	7	76
Guinea corn	<i>Sorghum vulgare</i> Linn	—	—	—	21
Bulrush millet	<i>Pennisetum typhoides</i> Rich	—	—	—	—

Nicol· Protein and Labile Methyl

769

APPENDIX (continued) AVERAGE DAILY FOOD CONSUMPTION (continued)

Foodstuff	Scientific name	Traders	Jews	Iskos	Daks
		g	g	g	g
ssava	<i>Manihot utilissima</i> Pohl	109	186	143	8
flour		102	92	172	—
starch		—	—	63	—
'fufu'		—	77	—	—
fresh tuber		300	63	633	—
am	<i>Dioscorea sativa</i> Linn	—	12	—	4
ocoyam	<i>Colocasia antiquorum</i> Schott	—	—	—	15
	<i>Plectanthus</i> sp	—	—	14	—
laffir potato	<i>Musa paradisiaca</i> Linn	17	13	—	—
plantain green	<i>Musa sapientum</i> Linn	22	19	—	—
banana	<i>Ipomoea batatas</i> Linn	—	—	13	15
sweet potato	<i>Vigna sinensis</i>	15	—	3	—
cow pea	<i>Artocarpus integer</i> Merr	—	—	2	—
jak fruit	<i>Arachis hypogaea</i> Linn	4	—	—	1
groundnuts	<i>Parkia filicoides</i> Welw	—	—	1	—
locust bean cake	<i>Cucurbita</i> spp	3	—	—	—
pumpkin seed	<i>Cocos nucifera</i> Linn	2	—	1	—
coconut kernel	<i>Pentaclethra macrophylla</i> Bth	—	—	—	—
oil bean kernel	<i>Irvingia gabonensis</i> Baill	1	—	1	1
	<i>Kola accuminata</i> Schott & Endl	3	1	—	—
	<i>Butyrospermum parkii</i>	—	—	—	45
sea nut husk		—	—	—	1
sea nut oil	<i>Allium cepa</i> Linn	4	—	—	2
mions	<i>Hibiscus esculentus</i> Linn	6	—	—	1
ikra fresh		—	—	—	1
ikra dried	<i>Hibiscus sabdariffa</i>	—	—	—	5
red sorrel fresh		—	—	—	—
red sorrel dried	<i>Cochorus</i> spp amaranths and others	12	—	—	—
green leaves	<i>Capsicum frutescens</i> Bl	—	—	—	—
Peppers		—	—	—	—
red		3	4	3	2
dry		2	1	3	—
fresh		5	1	2	—
green fresh		—	—	—	16
Baobab leaf fresh	<i>Adansonia digitata</i> B Juss	—	—	2	1
Baobab fruit		—	—	—	—
Tomato fresh	<i>Lycopersicum esculentum</i> Mill	4	—	—	—
	<i>Persia americana</i> Mill	26	—	—	—
Avocado pear	<i>Mangifera indica</i> Linn	20	—	2	45
Mango	<i>Detarium senegalense</i> Gmel	—	—	—	1
Taura		—	—	—	—
	<i>Psidium guajava</i> Linn	4	—	—	—
Guava	<i>Carica papaya</i> Linn	12	—	—	—
Pawpaw	<i>Citrus sinensis</i> sp	10	—	—	—
Orange	<i>Annona reticulata</i> Linn	7	—	—	—
Custard apple		—	—	—	—
Fish mixed estuary and river		23	78	18	—
dried		62	165	—	—
fresh		96	—	—	—
Beef		12	—	—	—
Mutton		—	—	—	—

APPENDIX (continued)
AVERAGE DAILY FOOD CONSUMPTION (continued)

Food-stuff	Scientific name	Traders	Law
Pork, fat		16	—
Goat		—	—
Monkey, dried	Various species	—	—
Rat meat	<i>Cricetomys gambianus</i>	—	—
Rat meat	<i>Thryonomys swinderianus</i>	—	—
Pangolin	<i>Manis longicaudata</i>	—	—
Porcupine	<i>Atherurus africanus</i>	—	—
Frogs' legs	<i>Rana mascarenensis</i>	—	—
African snail	<i>Archachatina</i> sp	3	—
Palm weevils	<i>Rhynchophorus phoenicis</i>	1	—
Snake meat	various species	—	—
Shrimps, dry		2	1
Prawns, fresh		—	4
Oysters, local		—	7
Liver		4	—
Eggs		10	—
Caterpillars		—	—
Sugar cane stem	<i>Saccharum officinarum</i> Linn	26	4
Palm oil	from <i>Elaeis guineensis</i> Jacq	36	27
Margarine, imported		3	—
Jam, imported		8	—
Sardines, tinned		3	—
Milk		—	—
sweetened condensed		5	—
evaporated		25	—
local, sour		—	—
Palm wine (ml)	from <i>Elaeis guineensis</i> and <i>Raphia vinifera</i>	120	103
Native beer (ml)	from sorghum vulgare and penisetum	—	—
"Tlicit gin" (ml)		20	107
Gin, imported (ml)		26	—
Whiskey (ml)		23	—
Brandy (ml)		13	—
Beer, imported (ml)		100	—
Tea		4	—
Coffee		3	—
Nut (Urbobo "ewoie")		—	1
Native salt		4	4
Imported salt		10	7

References

- 1 BEVERIDGE, J M C C & LUCAS & MARIAN K O'GRADY 1944 J
- 2 BROCK, J F & M AUTREY 1952 World Health Organization Geneva
- 3 CHANNON, H J, G T MILLS, & A P PLATT 1943 Biochem J 37
- 4 CHATFIELD, C 1949 Food and Agricultural Organization, Na Washington, D C
- 5 DAVIDSON, C S & G J GABUZDA 1950 New Engl J Med 243
- 6 FAGEL, R W 1943 J Nutrition 25, 441
- 7 FLETCHER, J P, C H BEST & O M SOLANDY 1935 Biochem J
- 8 JUKES, T H 1941 Poultry Sci 20 251
- 9 KOCK WESER D & H POPPER 1952 Proc Soc Exptl Biol Med

RELATIVE EFFECTS OF PROTEIN AND LIPOTROPIC SUBSTANCES IN THE TREATMENT OF NUTRITIONAL CIRRHOSIS IN RATS*

By Arthur J. Patek, Jr., Irvin C. Plough, and Margaret Bevans

College of Physicians and Surgeons, Columbia University, New York; Walter Reed Hospital, Washington, D. C.; Goldwater Memorial Hospital, New York

It is established that rats fed diets low in protein and deficient in lipotropic substances develop fatty changes and cirrhosis of the liver. It has also been shown that the addition of choline and methionine to these diets prevents development of the lesions. Fewer studies have dealt with the treatment of nutritional cirrhosis once it is fully developed.

The first reports on treatment of dietary cirrhosis in the rat were by Lowry, Daft, Sebrell, Ashburn, and Lillie.^{1,2} After producing cirrhosis in weanling rats with a diet containing 4 per cent casein, liver biopsies were performed, after which the animals were placed in two therapeutic groups: one receiving the basal diet plus 40 mgm. of choline daily, the other receiving a high casein diet containing either 30 or 50 per cent casein. In a short study, animals were sacrificed at intervals up to 42 days and, in a longer study, at intervals up to one year. Comparison was made with the biopsies. In both groups, the addition of choline to the basal diet or change to a higher casein diet brought about disappearance of fat regenerative changes in liver cells, but no apparent decrease in the amount of connective tissue.

Sellers, Lucas, and Best³ tested various dietary regimens on young rats with cirrhosis of the liver induced by carbon tetrachloride poisoning. After a moderate degree of cirrhosis was produced, a representative sample was sacrificed for control studies, the remainder being placed on the test diet for 12 to 13 weeks. In these experiments, the addition of choline, of methionine, or an increased proportion of casein brought about disappearance of fat regenerative changes in liver cells, and loss of connective tissue as well. These effects seemed to be equally effective. It was concluded, therefore, that the regenerative effects were attributable largely to lipotropic substances.

In a more recent report by Gyorgy and Goldblatt,⁴ employing a large number of rats, doubt was thrown on the effectiveness of lipotropic substances, particularly in advanced stages of the disease. Because of the high mortality involved, biopsy technique was abandoned, and their observations were based largely upon sampling after 75 to 150 days of treatment. In those rats with moderate degrees of cirrhosis, lipotropic substances seemed to have a curative effect, whereas in more advanced cirrhosis, the addition of these substances to the basal diet was much less effective. Best results were obtained by a combination of casein and liver extract.

The problem was re-examined because of the divergent results. In the experiments to be described, nutritional cirrhosis was produced in the rat on a diet patterned after that of Daft, Sebrell, and Lillie.⁵ An attempt was made

* Permission has been granted by the Journal of Experimental Medicine to reprint TABLES 1, 2, and 3 of this report.

protein and of lipotropic factors separately and case process. These studies suggest that high urative effects greater than those attributable to choline and methionine.

and biopsy technique, despite the attendant mortality.

and dietary supplements of penicillin and vitamin C. The animals were permitted to feed *ad libitum*.

The plan of the experiment is shown in TABLE 2. Choline was mixed with the diet in a proportion of 0.5 per cent. The average daily consumption per rat was 40 mgm of choline. Methionine was added at a level of 0.8 per cent. The average daily consumption per rat was 64 mgm. This was estimated to be equivalent to the methionine content of the 30 per cent casein diet.

Results The caloric intake was essentially the same for those on the 4 per cent and 30 per cent casein diets, but their rates of growth varied markedly,

TABLE 1
COMPOSITION OF EXPERIMENTAL DIETS

Low-protein		High protein	
	per cent		per cent
Vitamin free casein	4.0	Vitamin free casein	30.0
L-cystine	0.5	L-cystine	0.5
Wesson oil	5.0	Wesson oil	5.0
Salt mixture	3.0	Salt mixture	3.0
Corn starch	87.5	Corn starch	61.5

TABLE 2
PLAN OF EXPERIMENT

Group no.	Preperiod (16-19 wks.)	Treatment period (15-19 wks.)
1	4 per cent casein diet	Biopsy 4 per cent casein diet + choline + methionine
2	4 per cent casein diet	Biopsy 30 per cent casein diet + choline
3	4 per cent casein diet	Biopsy 30 per cent casein diet
4	4 per cent casein diet (control)	Biopsy 4 per cent casein diet
5	30 per cent casein diet (control) + choline	Biopsy 30 per cent casein diet + choline

those on the 30 per cent casein diets showing a sharp acceleration of growth. Unexplained.

30 per cent

unsaturated

dermatitis was but partially arrested. Of 143 rats employed, 69 died during the foreperiod. In this early stage, death was due chiefly to hemorrhagic cortical degeneration of the kidneys, presumably from choline deficiency. Others died within one week of the diagnostic biopsy. There remained 74 suitable for testing various therapeutic diets.

During the period of treatment, there was a sharp reduction in death rate in all groups, once the animals survived a critical period of about two weeks. The death rate was higher in those fed the 4 per cent casein diet supplemented with choline and methionine than in those fed 30 per cent casein diets.

Comparison was made of histologic sections obtained at the time of biopsy and at autopsy. Only those specimens were compared in which animals received treatment for at least 10 weeks. Sixty animals satisfied this criterion.

The results are shown in TABLE 3. The tabulation is restricted to fatty infiltration and changes in connective tissue, since other changes are difficult to compare in a quantitative fashion. Certain trends seem evident. For example, in the 30 per cent casein group, fibrosis appeared to increase in rats on the 4 per cent casein diet, despite the addition of choline and methionine. In contrast, those rats whose diets were changed from 4 to 30 per cent casein showed either moderate regression (group 2) or arrest (group 3) of the process. In this small group, it is doubtful whether the differences between

per cent casein, with or without added choline. Fibrosis appeared to increase in rats on the 4 per cent casein diet, despite the addition of choline and methionine. In contrast, those rats whose diets were changed from 4 to 30 per cent casein showed either moderate regression (group 2) or arrest (group 3) of the process. In this small group, it is doubtful whether the differences between

TABLE 3
HISTOLOGIC APPEARANCE OF LIVER AS DETERMINED FOR SPECIMENS AT BIOPSY AND AUTOPSY*

Group No			Fatty infiltration		Connective tissue	
			Biopsy	Autopsy	Biopsy	Autopsy
1	4 per cent casein + choline + methionine (18 rats)	Absent	0	4	2	2
		Minimal	3	10	8	3
		Abundant	15	4	8	13
2	30 per cent casein + choline (21 rats)	Absent	2	10	1	7
		Minimal	8	11	13	7
		Abundant	11	0	7	1
3	30 per cent casein alone (12 rats)	Absent	0	7	1	6
		Minimal	3	4	6	5
		Abundant	9	1	5	0
4	4 per cent casein control (4 rats)	Absent	0	0	1	0
		Minimal	0	0	2	4
		Abundant	4	4	1	5
5	30 per cent casein control (5 rats)	Absent	5	5	5	0
		Minimal	0	0	0	0
		Abundant	0	0	0	0

* Only those specimens are compared in which animals received dietary treatment for 10 or more weeks.

amino acid composition of casein. Such amino acid mixtures appeared to be as effective in promoting repair of the liver as any of the diets employed above.

Evaluation of results is always difficult, and comparison with other studies is hazardous because of different experimental conditions. There are several possible explanations. Possibly methionine contained in the 30 per cent casein diet is more effectively utilized than when it is added as a supplement to the low casein diet. Possibly other amino acids play a rôle in the reparative process.

In conclusion, within the conditions of these experiments, the studies suggest that the feeding of a diet containing 30 per cent casein is adequate for the test animal.

References

1. LOWRY, J. V., F. S. DAFT, W. H. SEBRELL JR., L. L. ASHBURN, & R. D. LILLIE. 1941. Public Health Repts. U.S.P.H.S. 66:2216.
2. LOWRY, J. V., L. L. ASHBURN, & W. H. SEBRELL JR. 1945. Quart. J. Studies Alc. 6:271.
3. SELLERS, E. A., C. C. LUCAS & C. H. BEST. 1943. Brit. Med. J. 1:1061.
4. GYÖRGY, P. & H. GOLDBLATT. 1949. J. Exptl. Med. 90:73.
5. DAFT, F. S., W. H. SEBRELL JR., & R. D. LILLIE. 1941. Proc. Soc. Exptl. Biol. Med. 48:228.

Discussion of the Paper

DOCTOR P. GYÖRGY, PHILADELPHIA. It is too much to ask to expect identical results with beneficial prophylactic measures in the therapy of the same condition, such as cirrhosis. In prophylactic experiments, the effective factors should and will prevent the development of the major specific metabolic and anatomical changes. Therapeutic efforts, on the other hand, have to deal with the arrest or even regression of already existing metabolic and anatomical disturbances. For instance, in the case of cirrhosis, the progress of fibrosis has to be checked or even reversed, in addition to the repair of all other concurrent pathologic manifestations. Furthermore, in cirrhosis, not only the liver but often other organs as well, such as the kidney and endocrine organs, among the latter especially the gonads, are found to be involved in the overall disease. In decompensated liver disease, high protein diet may even be injurious just as exercise may be harmful in decompensated heart disease. Protein, or hypotonic substances may be beneficial in the further progression of the disease and perhaps in the recovery stage.

PROTEIN METABOLISM IN PATIENTS WITH CIRRHOSIS OF THE LIVER*

By George J. Gabuzda, Jr.† and Charles S. Davidson

Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard) Boston City Hospital and Department of Medicine Harvard Medical School Boston Mass

The pathogenesis of hepatic cirrhosis is, in some measure, related to protein malnutrition. Contrariwise, established liver disease may affect nutrition including the metabolic observations relating to cirrhosis of the liver.

Concerning this aspect of nutrition in liver disease, and a presentation of data previously reported. The presentation of data derived primarily from laboratory is done with a full realization of the valuable contributions of others many of which have been cited in previous publications.

The data presented were obtained from the study of patients in the Thorndike Metabolic Ward where specialized dietary and nursing are available. All of the patients studied had advanced cirrhosis of the liver associated with chronic alcoholism and an inadequate dietary intake. They presented obvious clinical and laboratory evidence of parenchymatous liver disease in varying stages of activity and chronicity. In many instances the clinical diagnosis was confirmed by needle biopsy of the liver, or at necropsy.

All of the patients were allowed *ad libitum* ambulation on the ward, not being confined strictly to bed. None presented clinical or laboratory evidence of cardiac or renal disease, nor was any study complicated by vomiting, diarrhea, gastrointestinal bleeding or febrile illness. Although anorexia may be a prominent symptom of liver disease, this factor does not influence the metabolic data presented since the patients were maintained on constant intake of food as indicated. The methods of metabolic study, the various analytical procedures, and the clinical laboratory tests involved have been described in previous publications.

Consideration of the effect of well established cirrhosis of the liver upon the metabolism of protein is presented in the following sequence: (1) the metabolism of dietary protein, (2) the utilization and excretion of amino acids, (3) the untoward consequences of the administration of certain nitrogenous substances, and (4) finally an evaluation of these data as they apply to the dietary management of patients with this disease.

1 Protein

That digestion and absorption of ingested protein is normal in patients with cirrhosis of the liver is indicated in FIGURE 1, which demonstrates that nitrogen excretion is not increased above normal over a range of protein intakes of from 0 to 100 gm daily. For example the patients ingesting 10 gm

* This investigation was sponsored in part by the Commission on Liver Disease, Armed Forces Epidemiological Board, with support in part by grants from the Office of the Surgeon General, United States Army, Washington, D. C. and in part from Merck and Company, Rahway, N. J., to Harvard University.

† Welch Fellow in Internal Medicine of the National Research Council.

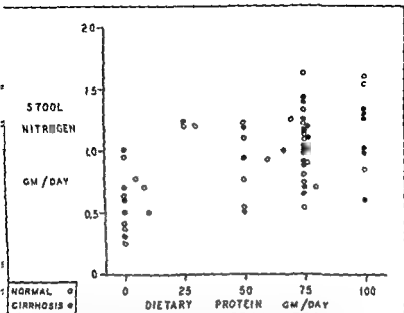


FIGURE 1. Stool nitrogen excretion in patients with cirrhosis of the liver and in normal subjects at various levels of dietary protein intake.

of protein daily excreted from 0.7 to 1.4 gm. of fecal nitrogen daily the normal subjects from 0.5 to 1.6 gm. daily ranges of values which are comparable in the two groups. The analyses of feces for nitrogen were made on from one to five

of absorption and metabolism of protein in severe cirrhosis¹

During studies designed primarily to elucidate the role of dietary factors on the course of cirrhosis²⁻⁴ the urinary nitrogen excretion was determined during periods in which patients ingested food providing calories but essentially devoid of protein nitrogen. The minimal urinary nitrogen excreted under these circumstances provided evidence concerning the rate of breakdown of endogenous protein. These data are presented in TABLE I. Three of the patients were given diets containing 0.7 to 2.1 gm. of nitrogen and providing 2500 to 3500 calories daily as carbohydrate and fat. These patients excreted from 2.3 gm. to 4.7 gm. of urinary nitrogen daily. Stool nitrogen excretion was about 0.6 gm. daily. Four other patients receiving 1600 calories as glucose solution orally and from 0.2 to 0.3 gm. of nitrogen daily excreted 2.1 to 4.1 gm. of urinary nitrogen daily. These values for urinary nitrogen excretion under conditions of minimal nitrogen intake indicate that the catabolism of

TABLE 1
CIRRHOSIS OF LIVER MINIMAL NITROGEN EXCRETION

Patient	Days of study	Calories daily	Nitrogen gm per day (average)			
			Intake	Urine	Stool	Balance
J S	7	3500*	2.1	4.7	0.5	-3.1
M P	11	2500*	0.3	2.7	0.7	3.1
F C	13	2500*	0.7†	2.3	0.6	5.1
J Mc	10	1600**	0.3***	2.9	0.6†	3.2
H C	4	1600**	0.2***	2.1	0.6†	2.5
S C	5	1600**	0.3***	2.5	0.6†	-2.8
W E	5	1600**	0.0	4.1	0.6†	4.7

* Fat and carbohydrate

** Glucose

† The nitrogen contained in choline chloride given orally during days 3 to 10 inclusive is included in the value

* The nitrogen was contained in choline dihydrogen citrate given orally daily

† Estimated

'endogenous' nitrogen was not accelerated. Further, the ingestion of 40 grams of glucose (20 per cent solution in water or in saline) provided 250 calories to prevent the utilization of significant quantities of nitrogen to the energy requirement under the conditions of the study.

The data to follow concern the utilization of dietary protein by patients with cirrhosis of the liver. Although many studies indicate that patients can

can be attained with moderate intakes of dietary protein suggests that patients are able to utilize dietary protein and that they are protein depleted. All patients ingesting four grams or less of nitrogen daily are in negative nitrogen balance. All patients receiving 12 gm or more of nitrogen daily are in positive nitrogen balance. Most patients given 8 gm of nitrogen daily are in positive nitrogen balance. It might therefore be conservatively stated that most patients with cirrhosis uncomplicated by other disease will maintain nitrogen balance if provided with daily diets containing 12 gm of nitrogen (56 gm of protein) and adequate calories. These data indicate that even the exceptional patients with the highest requirement for protein would maintain nitrogen equilibrium if given about 9 gm of nitrogen daily (56 gm of protein) although most patients require less than this amount.

That protein intakes of 75 gm daily will maintain positive nitrogen balance in patients with severe liver disease is further indicated by nitrogen balance

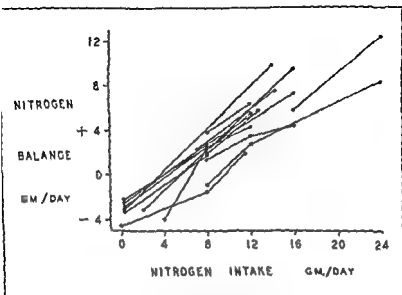


FIGURE 2 Protein requirement of patients with cirrhosis of the liver for maintenance of nitrogen balance

data obtained by the study of 16 additional patients with ascites who were given diets providing adequate calories and minimal intakes of sodium for study periods of from 15 to 30 days each. A summary of the data obtained from these studies is presented in TABLE 2. Nitrogen balances in this group of patients ranged from +1.5 to +6.8 gm. daily, and averaged +3.8 gm. daily, none being in negative balance under the conditions of study. Thus, the protein requirement of these patients for the maintenance of nitrogen equilibrium or positive nitrogen balance may be greater than for normal individuals but is low enough to insure positive nitrogen balance when 75 gm. (or even 56 gm.) of protein is given daily when adequate calories are also provided. "High" protein intakes were not necessary to achieve a positive balance.

The data presented do not indicate that there is maximally efficient utilization of dietary protein by patients with liver disease. Comparison of these patients to equally undernourished individuals without liver disease with regard to the efficiency of utilization of protein awaits some means of adequately evaluating an individual's state of protein nutrition. Our clinical impression has been that, nutritionally, it is more difficult to rehabilitate patients with chronic cirrhosis of the liver than patients who are undernourished due to starvation. The problem, however, has been approached in another manner. Selected patients with evidence of severe active liver disease were maintained on constants diets, and urinary nitrogen excretion was followed for 40 to 50 days during which progressive improvement, as judged clinically and by laboratory tests, was manifest. A progressive decrease in the urinary excre-

TABLE 2

NITROGEN BALANCE SIXTEEN PATIENTS WITH CIRRHOSIS WITH ASCITES, 15-30 DAYS
PER PATIENT, 2500-3500 CALORIES PER DAY

	Nitrogen gm per day			
	Def	Urine	Stool	Balance
Range	10.8-13.8	4.5-8.9	1.0-1.5	+1.6-6.1
Average	12.3	7.5	1.0	+3.8

tion of nitrogen was evident. The data obtained from the study of two patients in this manner are presented in FIGURE 3. These studies suggest some defect in the utilization of dietary protein in the presence of active disease and a more efficient utilization with improvement.

2 Amino Acids

The plasma concentration and the daily urinary excretion of alpha amino nitrogen were normal (TABLE 3), and the quantity of each of the ten 'essential' amino acids excreted in the urine by patients with cirrhosis of the liver has been found not to differ markedly from that observed in normal individuals maintained on comparable diets.^{4, 5} The most marked deviations from the normal noted were an increased excretion of methionine and of tryptophan and a decreased excretion of isoleucine. The increased excretion of these two essential amino acids was not considered to be nutritionally limiting nor to affect the patients' requirements for them. This is substantiated by the failure of dietary supplements of methionine (and choline) to enhance nitrogen balance in patients with hepatic cirrhosis.⁶ However, the failure to observe gross deviations from the normal pattern of urinary amino acid excretion does not imply that the intermediary metabolism of each of the amino acids is entirely normal.

In order to define further any abnormality in the urinary excretion of amino acids by patients with cirrhosis two additional methods of study were employed.⁷ One of these, designed to determine the effect of increases in dietary protein intake upon the urinary excretion of amino acids, demonstrated that increases in protein intake did not result in the urinary wastage of amino acids. During the other, the effect of the severity of the liver disease upon the urinary excretion of the ten 'essential' amino acids was noted by observing the alterations in their urinary excretion which occurred concomitant with clinical improvement in patients provided with adequate and constant diets. Progressive decreases in the urinary excretion of these amino acids were noted, suggesting a more efficient utilization of amino acids with improved liver function. The conclusion drawn was that the activity of the patient's disease influenced the urinary excretion of these amino acids more than did alterations in the dietary protein intake. These data indicating a decreasing urinary excretion of amino acids with clinical improvement, and the studies mentioned above which indicate a decreasing urinary excretion of nitrogen under similar conditions of study suggest the presence in patients with severe liver disease of an

TABLE 3
PLASMA AND URINE ALPHA AMINO NITROGEN

	Patients with cirrhosis	Normal subjects
Plasma, mgm /100 ml		
No pts	12	35
Range	2.7-4.6	2.9-4.5
Average	3.9	3.7
Urine, mgm /24 hr		
No pts	15	10
Range	84-258	118-109
Average	171	152

TABLE 4
PLASMA ALPHA AMINO NITROGEN (MG PER 100 ML) FOLLOWING INTRAVENOUS INT. OF
300 ML. 15% PROTEIN HYDROLYSATE

	Before infusion	After infusion	
		1 hr	4 hr
Patients with cirrhosis (8)			
Range	2.7-4.3	6.3-12.6	3.4-4.1
Mean	3.7	8.3	5.5
Normal subjects (5)			
Range	3.5-4.5	5.0-9.6	4.2-4.9
Mean	4.0	7.3	4.3

or other digestive disturbances may require the provision of nutrients by parenteral feeding. Since the patients with damaged livers might not metabolize amino acids normally when given parenterally, the advisability of administering amino acids to these patients has been questioned. For these reasons studies were undertaken to evaluate the tolerance, excretion, and therapeutic value of the intravenous administration of solutions of amino acids or protein hydrolysates to patients with cirrhosis of the liver. Their tolerance to the rapid intravenous infusion of a protein hydrolysate (300 cc, 15 per cent solution) was not grossly abnormal (TABLE 4). The clearance of amino acids from the blood by the patients was only slightly delayed as compared to normal individuals. Furthermore, in one study, a progressive rise in serum alpha amino nitrogen concentration did not occur in a patient receiving 100 gm. of amino acids intravenously daily for 18 days.*

Further evidence that intravenously administered amino acids are metabolized comes from the nitrogen balance studies over 2 to 4 weeks of 16 patients with active cirrhosis of the liver, given a solution of amino acids intravenously daily as the sole source of nitrogen, while they were given a

* Each of the 16 patients was given a diet with adequate protein and did not occur.

In addition improvement in the liver disease, as evidenced clinically and laboratory tests of liver functions, was observed during this period. The pattern of the infused amino acids which were excreted into the urine by

that patients with liver disease are not able to remove ammonia adequately from the blood due, for example, to possible defects in the intermediary metabolism of glutamic acid

Thus, although the mechanism of the reaction noted in the patients with cirrhosis of the liver given the nitrogenous substances is not clear, the studies suggest that the administration of these materials may be harmful and offer additional evidence that a defect in nitrogen metabolism exists.

All patients given nitrogenous substances did not develop the uremic reaction and, although all patients studied presented evidence of severe liver disease, whether or not a patient had a reaction did not relate to liver function as measured by the usual tests. For example, only two of six patients in whom the dietary protein intake was increased to 150 gm daily developed uremic symptoms.¹⁰ Subsequent to this study, three additional selected patients have been observed who displayed neurological signs attributable to increased dietary protein intake. Even this incidence of reactions following increased protein is significant, since large quantities of dietary protein are frequently recommended in treatment. On the basis of the observations cited, the advisability of providing large quantities of nitrogenous substances and increasing dietary protein to patients with severe liver disease, especially those in hepatic coma, may be questioned.

Summary

Data have been presented which demonstrate that the absorption of nitrogen was normal in 28 patients with cirrhosis of the liver. Studies of urinary nitrogen excretion in seven patients given 1600 to 3500 calories and diets providing minimal intakes of nitrogen indicate that "endogenous" protein was metabolized at an accelerated rate. Each of 32 patients studied demonstrated a positive nitrogen balance when provided with 75 gm of protein daily. In fact, most of these patients maintained positive nitrogen balance, when provided with 50 gm of protein daily in a calorically adequate diet. For additional patients also maintained positive nitrogen balance when given adequate quantities of amino acid solutions intravenously as a sole source of nitrogen. "High" protein intakes were not necessary for patients with well-established cirrhosis of the liver to achieve positive nitrogen balance.

The urinary excretion of "essential" amino acids by patients with cirrhosis did not deviate grossly in pattern or in quantity from that observed in normal individuals given comparable diets or intravenous infusions of protein hydrolysate. The urinary excretion of amino acids was affected more by the severity of the liver disease than by the dietary protein intake. Following the intravenous infusion of amino acid solution their clearance from the blood was only slightly delayed in the patients. These data do not imply that the intermediary metabolism of each of the amino acids or of the nonessential amino acids is normal in patients with liver disease.

A defect in the utilization of ingested protein is suggested by the somewhat greater protein requirement for the maintenance of nitrogen balance in some of the patients than that for normal individuals by the decreases in urinary nitrogen and amino acid excretion observed with clinical improvement.

inally, by the untoward reactions noted following the administration of nitrogenous substances including dietary protein

The studies presented do not indicate to what extent the dietary protein deficit is related to the completeness or speed of recovery of patients with

with this disease

References

1. At
- effects of
J Clin
- 49 The
cretion of
n Invest.
- 6 GABUZDA, J. J., JR., R. D. ECKHARDT, & C. S. DAVIDSON. 1950. Effect of choline and methionine, testosterone propionate and dietary protein on nitrogen balance in patients with liver disease. *J Clin Invest* 29: 566-576.
- 7 LEWIS, J. H., F. H. L. TAYLOR, & C. S. DAVIDSON. 1947. Tolerance to intravenously administered protein hydrolysate in severe human liver cirrhosis. *Am J Med Sci* 214: 446-451.

TREATMENT OF INFANTILE CIRRHOSIS OF THE LIVER WITH ANTIBIOTICS

By P. Krishna Rao

Pathologist and Superintendent, Victoria Hospital, and Lecturer in Pathology, Un School, Bangalore, Mysore, India

Infantile cirrhosis of the liver is a peculiar condition found in Indian children. The disease commences insidiously between the ages of 5 and 18 months, which period the liver begins to enlarge gradually up to a size of four fingers' breadth with a comparatively small increase in the size. In the last stages of the disease, there may or may not be jaundice, but there is an invariable sequence. At the final stages, death is due to hemorrhage.

In my previous publication (1941), it was pointed out that the pattern of infantile cirrhosis is that of Laennec's (portal) type and that the disease falls into two stages. It is my belief that, in the early stage, there is involvement of the liver,

that, subsequently,

by *Escherichia*

authors has discussed the view that lack of vitamin B factors, especially in food, may be involved in producing cirrhosis of the liver. Bickel and Prescott have said that milk is not a good source of choline (1946).

In my previous paper (1951) on the treatment of infantile cirrhosis of the liver, it was shown that oral administration of choline and injections of streptomycin have proved successful. The object of the present paper is to give briefly a further note on Streptomycin and the effects of other antibiotics in the treatment of cirrhosis of the liver.

Streptomycin Many more cases have been treated with this drug since 1951 and the results have been uniformly good. In fact, this has become the treatment for infantile cirrhosis cases in this part of India. The usual dose is 4 to 5 gm, injected in daily doses of $\frac{1}{4}$ to $\frac{1}{2}$ gm, continued over a period of 15 to 20 days.

Aureomycin Four cases were treated with this antibiotic. Aureomycin spersoids were used throughout. One teaspoonful three times a day, a total quantity of 150 mgm. of Aureomycin HCl was the routine dose for the first cases, and advanced cases received 250 mgm. or more per day. Diarrhea, the usual complication, and the drug had to be stopped in the middle of treatment for one, two, or even three days, until the diarrhea was controlled. All the children died. Therefore, this drug has not proved useful in the treatment of infantile cirrhosis of the liver.

Terramycin The next antibiotic being tried is Terramycin. This is available in the form of drops, the powder being dissolved in the solvent supplied by the manufacturers. A daily dose of 600 mgm is administered in three

* Work is being carried out here in collaboration with Sri M. Bhargava Rao, a Fellow of the Research Board (Bombay) and Doctor K. P. Basu at the Indian Dairy Research Institute, Bangalore. The results will be published in course of time.

loses Six cases have been tried so far for the last six months. Marvelous results have been observed. In the case of four children with an early stage of liver enlargement (about 2 fingers), the liver came to normal size by the time 3 gm of the medicine were completed. Tonics containing choline methionine and vitamin B complex were given simultaneously as adjuvants. Even in two other cases which were advanced with jaundice and ascites the results were

Remarks

I am aware of the fact that the number of cases treated is very small to draw any definite conclusions. In accordance with my theory that *E. coli* is responsible for bringing about the cirrhotic changes in the liver I tried all the various antibiotics that have been proved to act against this bacteria

cirrhosis and as such a trial should be carefully conducted and reported

Summary

In this series of cases in addition to Streptomycin two new antibiotics Aureomycin and Terramycin have been tried in cases of infantile cirrhosis of the liver. Terramycin has been found to be very efficacious in curing the condition.

References

1. BICKNELL F & B. PRESCOTT 1946 The Vitamins in Medicine 1 112 Heinemann London
2. RAO P. K. 1941 Proc. Indian Acad. Sci. 14 310
3. RAO P. K. 1950 Indian Med. Gaz. 85(4) 150

METABOLIC AND NUTRITIONAL PATTERNS IN ALCOHOLISM

By Jorge Mardones

Instituto de Investigaciones sobre Alcoholismo Universidad de Chile Santiago Chile

The reasons for considering the nutritional and metabolic patterns in beer alcohol addicts as differentiated from those of nonalcoholic persons are two: the one based on clinical and the other on experimental viewpoints. The clinical reason is that certain deficiency diseases appear with significantly greater frequency in alcohol addicts than in nonalcoholics. These are liver cirrhosis, polyneuropathy and other forms of thiamin deficiency, and pellagra. The experimental reason is that it has been observed in rats and mice that when their diet lacks certain B complex vitamins they increase their voluntary ethanol alcohol intake, thus showing that nutritional factors can alter the appetite for alcohol.

In human beings, the nutritional conditions depend, on the one hand, on the characteristics of the appetite, and on the other, on the ability to satiate it. I use the term "appetite" not in the restricted sense of the desire to ingest food in general, but to eat certain foods.

Human appetite has a very complicated mechanism, and it differs in each person. Fundamentally, differences in the appetite are the result of genetic constitution, as well as of characteristics acquired by experience, education and pathological events. In their daily fluctuation, they are the result of physiological changes and pharmacological actions.

Thus, if a group of persons shows a nutritional pattern, it means that there are, in the constituents of the group, certain similitudes in the factors affecting nutritional conditions, namely, genetic constitution, experience, education, and appetite.

There may exist in the social classes of low economic level in certain countries in which a common factor of limited availability of foods is present. Obviously, examples of this nature can be multiplied.

In the case that concerns us now, however, in which we assume the presence of a nutritional pattern in a group, not as the result of a dietary survey, but because of the frequency of deprivation diseases, the problem increases in difficulties because of the appearance of new variables. In fact, the individual variations are now playing a new role since, with the same deficient diet, different individuals show differences in the development of pathological consequences. Such individual differences are produced particularly by genetic constitution and also by the occurrence of pathological conditions.

All the preceding considerations force us to conclude that, if thiamin deprivation symptoms are frequent in alcohol addicts, it means that the addict leads the patient to consume a diet low in thiamin or that a relationship of some other nature exists between addiction and thiamin deprivation. In fact, it is possible that alcohol addicts are more sensitive to thiamin deprivation.

that thiamin deprived persons are more likely to become alcohol addicts. *Musis mundis*, the same can be said concerning liver cirrhosis and low protein diet, as well as in the case of pellagra and nicotinic acid deficiency.

Let us now consider each of these points. There is no doubt that, during their so called "benders," alcohol addicts reduce their food intake to under-nutrition levels, and this may be the cause of deficiency diseases, but, on the other hand, we have to consider that undernutrition is frequent enough in certain populations so that a more even distribution of these diseases may be expected between alcohol and non-alcohol addicts. The same may be said of liver cirrhosis and low protein diet.

Obviously one question must be posed first, namely, does alcohol ingestion increase the sensitivity of individuals to these deficiency diseases?

Concerning thiamin, it has been established by Lowry, Sebrell, Daft, and

others, too soon to review this problem. In any case, it has not been proved

that, in the same time, favor both the development of alcohol addiction and the above mentioned diseases.

There is no doubt that the psychological factors which play a role in the pathogenesis of alcohol addiction are similar to those which induce other forms of mental trouble. It is therefore, necessary to assume that there are personal conditions favoring the influence of these psychological factors toward alcohol addiction.

It is now clear why it is important to review the experimental facts that convince us that other kinds of relationships can exist between nutritional conditions and habitual drinking of alcohol.

Richter and co workers, in papers appearing between 1937 and 1941 reported that when normal rats are free to choose between foods, they eat a balanced diet¹² and that they adapt the diet to the needs changed by experimental endocrine disturbances¹⁴ ¹⁵ or by vitamin deficiencies.¹⁷ Richter and Campbell¹⁸ on the other hand, reported that, under free choice, rats prefer a weak alcohol solution to tap water. Mardones and Onfray, in 1942,¹⁹ having in mind the fact that a disturbance of the carbohydrate metabolism between C_1 and C_2 compounds could increase the desire for alcohol, studied, with rats, the influence of a diet deprived of thiamin on the voluntary alcohol intake. Their first work showed that, in these circumstances, rats significantly increased the alcohol intake. If these rats received a thiamin supplement, they improved their weight and general appearance but they maintained the high level alcohol intake. If, however, they received a supplement of liver, meat, wheat germ, untreated yeast, the voluntary alcohol intake rapidly reached the normal level. Since the diet lacking thiamin contained autoclaved yeast, we claimed the presence of a new thermolabile factor of the B complex which we designated factor N. Subsequently my associates and I²⁰ reported that, if the supplement of thiamin was maintained for some weeks, the rats decreased the voluntary alcohol intake which reached the normal level in about twelve weeks, and that if normal rats were fed on a diet containing autoclaved yeast plus thiamin the voluntary alcohol intake was maintained at the normal level. On the other hand, rats fed a diet containing only ten pure vitamins of the B complex and showing a high level alcohol intake, decreased the alcohol intake in a similar way after supplementation of yeast, regardless of whether the yeast was autoclaved or not. The thermolabile factor present in yeast and liver responsible for the decrease of the alcohol intake in these conditions has been called factor N_1 . Actually, these findings show that factor N is composed of thiamin (thermolabile), and N_1 (thermostable).

In the meantime, Brady and Westerfeld² reported new facts along this line. These authors confirmed that rats fed on a diet deprived of all the elements of the B complex rapidly increased the voluntary alcohol intake, but they observed that these rats decreased it only temporarily after each continuous supplement of vitamins. On the other hand, they reported that the same diet that prevented the increase of the alcohol intake is unable to maintain it at a low level in rats that have reached a sizable intake. The authors suggested then that this fact means that "nonnutritional factors complicate the picture."

The facts reported by Brady and Westerfeld were explained later on. Beecher and co workers¹ showed that a single deprivation of riboflavin, pyridoxine, or pantothenate also increased the voluntary alcohol intake of rats and that it dropped suddenly when the specific deprivation was corrected. This fact led to the interpretation that the transitory drop observed by Brady and Westerfeld was the result of partial satisfaction of the deficiencies that produced the increase of the alcohol intake. On the other hand, we have reported²¹ that, if a sufficient amount of yeast supplement is given to rats with high lev-

ol intake, the decrease of the voluntary intake was permanent. Thus, second fact reported by Brady and Westerfeld is presumably the result of

■ substance present in liver of yeast that can decrease the alcohol intake

mesium level in blood. No biochemical reason has been postulated to aim the relation between these findings and the excessive alcohol intake. Recently Varela, Penna, Alcama, Johnson and Mardones²² have initiated a study of the carbohydrate metabolism in human alcohol addicts with the idea that a disturbance in it could explain why the energy of the alcohol is more

available to alcoholics than that of the carbohydrates. Their findings show that the glucose tolerance did not differ in the group of alcoholics from that of social drinkers and abstainers but that some differences in the two groups in the blood pyruvate curve and in the blood acetone curve after glucose ingestion are significant at the 2 per cent level. These results cannot yet establish the presence of a metabolic disturbance in alcohol addiction, but it encourages new studies along the same line.

After this paper was prepared, I became aware of some new facts. I wish to comment briefly as follows.

Recently Lester, Greenberg, Smith, and Wu⁶ reported a finding which interferes with the interpretation of the voluntary alcohol intake in rats. They observed that, if rats showing high level alcohol intake were allowed to ingest sucrose or saccharin solutions they decreased significantly the alcohol intake. It is not easy to interpret this fact since those rats had free access to water containing sucrose, which means that their appetite for this sugar was increased according as it was in solution or in crystalline form. On the other hand, the interpretation is complicated by the circumstances that the experimenters used showed a very high level alcohol intake, that the diet was low in carbohydrate, and that the rats were not actually depleted. In any case, these results would necessitate study of the effect of nutritional imbalance on the selection between an alcohol solution on the one hand and a sucrose solution on the other, instead of a dry diet containing crystalline sucrose.

Two other facts have been reported that are related to the problem under discussion. The first one was published by T. B. Sirnes⁷ of the Department of Pharmacology of the University of Oslo. He observed that rats with liver cirrhosis induced by treatment with carbon tetrachloride showed significantly higher voluntary alcohol intake, and that this increase was a consequence of the cirrhosis induced and not of other pharmacological effects of carbon tetrachloride. This shows that a liver lesion can induce an increase in alcohol intake.

The other fact is reported in this monograph by Olson and DuBois (page 889). They observed that, in the liver of rats with dietary necrosis, there is an important depression of the pyruvate oxidase activity.

All the facts analyzed in this talk induce us to believe that voluntary alcohol intake is increased when a difficulty in the utilization of the energy of the carbohydrates or fat is present and that this difficulty does not interfere with the utilization of the energy of the alcohol. They also induce us to suspect that, in some cases, the excessive ingestion of alcohol is not the cause but the consequence of nutritional or metabolic difficulties.

- LOWRY J W W H SPRELL, F S DART & I I ASHBURN 1942 J Nutrition 24 73
- MARDONES J A HEDERRA & N SEGOVIA 1949 Bol soc biol Santiago Chile 7 1
- MARDONES J & I ONFRAY 1942 Rev chilena hig y med prevent 4 293 11 148
- MARDONES J N SEGOVIA F ALCAVARO & A HEDERRA To be published
- MARDONES J N SEGOVIA & A HEDERRA 1947 1948 Bol soc biol Santiago Chile 4 121 5 27
- MARDONES J N SEGOVIA & A HEDERRA 1950 Bol soc biol Santiago Chile 7 82
- " " " " " " Quart J Studies Alc 14 1 20 481
- " " " " " " 31 507
- " " " " " " 31 50
- " " " " " " 131 639
- " " " " " " 133 Am J Physiol 122 734
- " " " " " " 28 179
- " J MARDONES 1953 Quart J Nutrition 30 127
- " " " " " " 1949 Proc Natl Acad Sci

THE GENETOTROPHIC CONCEPT—NUTRITIONAL DEFICIENCIES AND ALCOHOLISM

By Roger J Williams

University of Texas, Austin Texas

A full appreciation of the general and far reaching significance of the trophic concept^{1, 2} requires that we recast, if not revolutionize, our ideas regarding how we should use statistical methods in our attempts to solve biological and human problems

The revolutionary point of view which enters into this concept is in line with the current opinion of geneticists that every genotype determines its 'norm of reaction' to the environment³ and that no species can adequately be described in terms of a type specimen. It is also in accord with the idea of partial genetic blocks^{4, 5} which recent evidence has indicated to be the rule rather than the exception⁶

In the areas of physiology, psychology, biochemistry, and medicine, as well as in philosophy and the social sciences we have built into our thinking the concept of the *normal man* whom we regard as of paramount importance. We have set it as our supreme task to understand how this hypothetical creature functions. In the field of animal biology, the rat, the guinea pig or the salamander etc become the center of our attention. This is the view which is incompatible with the genetotrophic concept.

Biological variability is recognized by the biological fraternity as a more or less necessary evil. When it is extreme, it makes very difficult the establishment of norms. However by proper statistical treatment, its effects are smoothed out and we are as it were back on the right track again. The tacit assumption is made that the normal man is normal through and through, and that abnormalities are to be observed only in those who belong in the category of the abnormals. This assumption is manifestly false, as will be made clear in our discussion. Nevertheless it is commonly accepted and underlies the firmly entrenched idea of the normal man (or rat or guinea pig) which appears to be a guiding concept for most monographs, advanced books and research publications in all of the areas of biological science.

This attitude which centers its attention on those supposed individuals whose attributes always lie within the normal range is based in part upon another idea which is firmly entrenched in much biological thinking namely that on the microscopic level the cell is the center of our interest. If we say we can ascertain how the cell is constructed and how it works we will have gone a long way toward understanding biology.

The fundamental reason why we construct the 'normal man' in our minds is so we can develop generalizations which in a sense are the essence of science. Scientific progress in the area of biology as well as in other areas may be measured in terms of the number and scope of valid generalizations which exist the more far reaching the generalizations the more advanced the science. It is my broad thesis however that biological variability in the human

inevitable, but those which are made without regard for human variability are very likely to be premature and completely misleading. Leo Loeb,⁷ who has devoted a lifetime of study to variability, has said that individuality is most highly developed in the human species, and this observation alone should make us alert to its possible implications.

Let us look hastily at a number of human problems which naturally fall in the hitherto undeveloped field of *applied human biology*. Consider sex crimes for example. Can one be at all sure that "the normal human being" commits these, and that *deviates* in the area of sex physiology are not responsible? If we admit that this is an area where deviation may be important, how about the broader problem of sex adjustments in which all human beings are concerned? Can anyone say *a priori* that these adjustments characteristically involve 'the normal human' and that the enormous variability which Kinsey's report brought to light (much of which is probably basically inborn) has nothing to do with these adjustments? Marriage and divorce, and hence our whole social structure, have deep biological roots, and it seems probable that the enormous variability in sex urges, inclinations, and predispositions are of paramount importance and that the very deviations (which may be found in any of us) merit serious study instead of being smoothed away by statistical techniques.

I should like to interpose here another reason why 'man' or 'the normal human being' has in the past been so much the center of interest. We should like to think that, at the beginning of life we have an equal start and that we are the makers of our own destinies. If many of our problems arise out of our hereditarily controlled variation, we reach an 'apparently gloomy conclusion' as Professor Dunn⁸ puts it, 'toward which all results of heredity seem to be driving us,' namely, that our shortcomings and hence our difficulties are fore-ordained and hence irremediable. This conclusion is by no means a valid one, and the genetotrophic concept points the way very significantly as we shall see later, toward its demolition or at least its radical modification.

Consider next, if you will, the broad problem of health as a field of applied human biology. How many so called metabolic diseases have their roots in inborn metabolic peculiarities? We know for example that diabetes does and, on the basis of present evidence, it seems probable that other metabolic diseases

answerable question

If we concede that inborn variability may be important in connection with metabolic diseases what shall we say about infective diseases? Professor Gowen of Iowa State College has recently shown⁹ by combining a genetic study of mice with a study of their responses when challenged with tuberculosis germs of varying virulence that the problem is three dimensional as is shown in

FIGURE 1

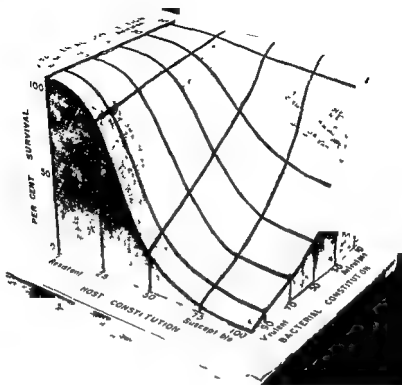


FIGURE 1

It would be difficult to controvert the idea that *all* infective diseases are three dimensional in the same way

There are numerous other diseases not classified as metabolic or infective for which the etiology has been for decades completely unknown or at least obscure. Among these are cancer, allergies, mental disease, alcoholism, dope addiction, and many so called degenerative diseases.

In all of these diseases a common fact may be observed. The diseases attack only *certain individuals* while others in the same environment escape completely. One member of a community may have cancer at a relatively early age. Another, who has the same occupation, eats the same food, breathes the same air, drinks the same water, lives in a similar house, is exposed to the same sunshine and weather, may live to be 90 years of age with no threat of the disease. A similar statement applies in the case of allergies. Some people are unfortunate, others are fortunate. One member of the community may develop mental disease, while his fellows, who have perhaps just as many harassments and frustrations, remain well balanced. Two individuals in the same environment can drink alcoholic liquors to the same extent over a period of

years. One may, however, become an alcoholic, an uncontrolled drinker, while the other may remain a heavy but well-controlled drinker. When different individuals under hospital conditions are repeatedly given morphine, most of them escape aftereffects, but some, for no known reason become addicts. Heart disease is a type of degenerative disease which attacks certain individuals and not others, and there is apparently no environmental influence which determines who will be the victim.

What hypothesis regarding these diseases can match for importance the one which postulates that in every case the individual's predispositions rest upon biological deviations from the respective norms? "Why is the 'normal human being' subject to allergy? Why does the 'normal human being' become an alcoholic? Why does the 'normal human being' have heart disease?" These, from the standpoint of our discussion are foolish and misleading questions. Far more in order are the questions "What deviations do we find in those subject to cancer? What metabolic peculiarities make one subject to allergy? What is different about certain persons which makes them become alcoholics? What kinds of deviations do we find accompanying specific types of heart disease?"

It seems probable that learning about 'normal' characteristics is only the first step. We must learn more and more about deviations and in order to do this, we must give them serious and attentive study. We must not eliminate them by statistical treatment. Instead we must search for them and discover their nature. We must scrutinize them. We must find out about their frequencies and be continuously aware of their existence and their possible influence. I believe convincing evidence is actually at hand which shows that, mathematically and scientifically speaking no such thing exists as a human being who is normal through and through. An individual may be 'normal'

ge, Kirk, Lewis,
(1) lipid phos

plus, (2) free cholesterol, and (3) lipid amino nitrogen. From FIGURE 2 and the accompanying tabulated data in the original article it is evident that four individuals lie below the normal range (at the 95 per cent level) numbers 5, 18, 29, 67, and one individual, number 63, lies above this range. From FIGURE 3 and the accompanying data it is clear that there are two individuals below the normal, 51, 56, and three above 24, 42, 61. But these are not the same individuals that appeared abnormal in FIGURE 2. They constitute a completely new set of "abnormals." From FIGURE 4 and the accompanying data one individual number 13 is below the normal range and two numbers 35 and 51, are above it. Here appears another new set of abnormals entirely different from the others.

It does not take very much imagination to conclude that if these 67 indi

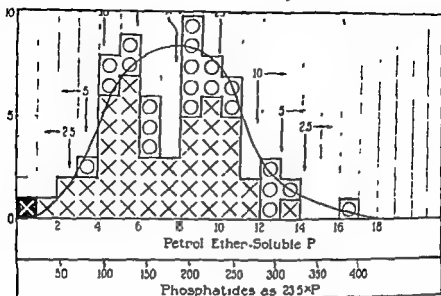


FIGURE 2

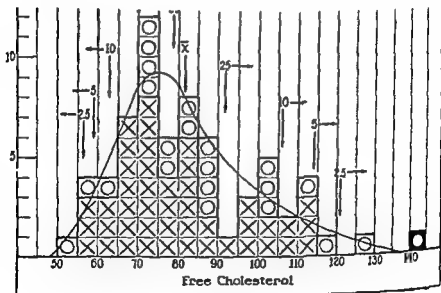


FIGURE 3

viduals were studied further and if the series of measurements were extended to include many diverse items, all of the 67 would be found to be "abnormal" in some respect. A substantial number of deviations are probably present in the makeup of each of us. Deviation is universal and, if an individual should turn up who is in the "normal range" with respect to every item in his makeup, he would be a most extraordinary and, in fact, a most "abnormal" individual.

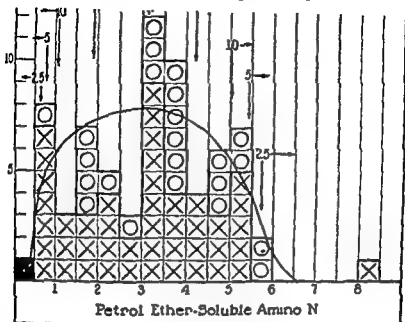


FIGURE 4

This point of view is further borne out by a consideration of the individual metabolic patterns¹¹ which we have studied at the University of Texas. Twelve individuals were measured several times with respect to 31 items: 5 taste thresholds, 12 salivary constituents, 14 urinary items. FIGURE 5 is a drawing, using polar coordinates, representing the hypothetical case of an individual who is exactly average with respect to every one of the 31 items. In FIGURES 6-17 are the corresponding diagrams for each of the twelve individuals, using as the basis an average of the values obtained from each one.

In the pattern of *each* individual, there are from 2 to 4 items in which their distinctive values are one tenth or less that of the corresponding average values. In other words, the average, taken as a norm, is of the wrong order of magnitude for some items in the case of *every* individual in the group. In this study, every finding points to the nonexistence of the human being who is "normal" in all respects.

In considering the effects of environmental influences on human beings—food composition, temperature, light, infective agents, and drugs which may combat them, altitude, noise, housing and clothing, home and social influences, etc.—we are prone to think in terms of their effects upon the hypothetical "normal human being," and to disregard the biological variation which makes each of us a mosaic in which greater or lesser deviations are the rule rather than the exception.

In the field of nutrition, emphasis on the concept that people, as a rule, are



FIGURE 5

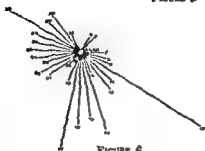


FIGURE 6

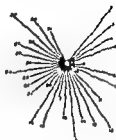


FIGURE 7

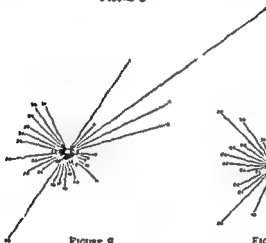


FIGURE 8

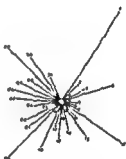


FIGURE 9

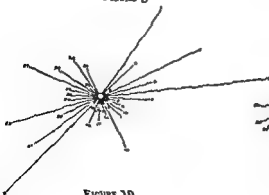


FIGURE 10

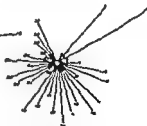


FIGURE 11

Figures 5 17
6 Uric acid 7
15 Serine, 16 G
21 Gonadotropin
Creatinine 27



FIGURE 12

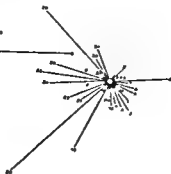


FIGURE 13

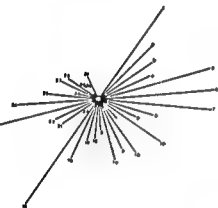


FIGURE 14



FIGURE 15



FIGURE 16

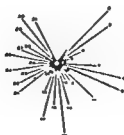


FIGURE 17

consistently normal is particularly pertinent. Indeed, if the population consists essentially of normal human beings who have substantially identical nutritional needs, then the genetotrophic concept about which I am to speak is invalid and must be completely disregarded.

In the light of modern biochemical genetics, however, this uniformity in nutritional needs cannot exist. Genes in a sense beget enzymes, and enzymes require substrates in the form of foods and the metabolites derived from them. Since the assortment of genes is distinctive for each of us, we must each have a distinctive pattern of enzymes and enzyme systems of varying efficiencies, and it follows, therefore, that our substrate needs are not the same, quantitatively speaking. The widespread prevalence of partial genetic blocks¹ or their equivalent, makes it reasonable to suppose the nutritional needs of each of us for calcium, for leucine, for vitamin A, for riboflavin *etc.*, are distinctive. The variabilities may be large or small, but they are inescapable. There is reason, I believe, to suspect that the deviations are much larger than has traditionally been thought. They certainly have not been studied sufficiently so they can be dismissed as inconsequential.

With this discussion as a background, it becomes easy to indicate the meaning of the genetotrophic concept. It is simply that some individuals because

as a result. If, however, such individuals are furnished an extra supply of the items which they individually need, their difficulties should disappear and their status should be as though the basic genetic peculiarities did not exist.

We have postulated that numerous diseases of which the etiology is obscure may have genetotrophic origins.^{1, 2} Such diseases by definition are of genetic origin but are also nutritional (trophic) in nature and hence ideally may be eliminated by nutritional means.

A clear cut case where the genetotrophic concept appears to apply is the disease alcoholism which I wish to discuss briefly. Actually, the genetotrophic concept grew out of our experimental findings while we were studying the problem of alcoholism and hence in this discussion I have in a sense placed the cart before the horse. This has been done deliberately, however, because the genetotrophic concept is much more far reaching in its implications than anything that could be said about alcoholism, and I hope that the subject of alcoholism will not crowd the broader aspect of the genetotrophic concept out of your thinking. I do wish to make it clear, however, that our theorizing

We did, however, after a careful survey of the literature, come to the conclusion that potential alcoholics probably have inborn differences which needed to be sought out.

Our first attack consisted in a prolonged and detailed study³ of the metabolic patterns of a group of alcoholics who were not drinking at the time and of a corresponding control group. The items investigated were more numerous,

TABLE 1

TABULATION OF HIGH AND LOW CHARACTERISTICS IN ALCOHOLICS IN ORDER OF DECREASING SIGNIFICANCE

High	Low
Sodium (S) (99)	Gonadotrophin (U) (96)
Hippuric acid (U) (99)	Citrulline (U) (95)
Uric acid (U) (95)	
Uric acid (S) (95)	
Thiamine (U) (94)	
Citrate (U) (92)	
Magnesium (B) (92)	
Pigment/creatinine (U) (91)	Phosphorus (B) (90)
Weak acids (U) (88)	Taurine (U) (88)
Sodium chloride (T) (88)	<i>Bacillus subtilis</i> (P) (88)

Numbers in parentheses are P values for difference between alcoholics and controls (S = saliva U = urine, B = blood serum T = taste threshold P = phagocytic index)

but similar to those of -
included
noted that t
n -

but similar to those of -
included
noted that t
n -
but several evidences bearing upon it. Identical twins (FIGURES 16 and 17) were found to have patterns very much alike¹¹ indicating that they do not change materially the all babies living largely on Experimental animals ex-
very distinctive excretion patterns when on identical diets⁷ and an extensive study of several closely inbred strains of rats shows clearly that these patterns have a genetic origin¹². All these facts point to the conclusion that the alcoholics probably were deviates of the sorts indicated by their patterns before they began excess drinking.

The other
the work of
the existen
initial work

colony
wet and
other 10

very similar to this one had been done before by Richter²³ and Mardones,¹⁴ Brady and Westerfeld²⁵ and others. The distinctive element in our study was that we ignored averages and concerned ourselves with the drinking behavior of individual rats. We found a high degree of individuality. Some rats drank considerable quantities of alcohol the first time it was offered and every day thereafter. At the other extreme some rats were teetotalers. One rat in the original group drank just a little alcohol every day as long as the

experiment lasted. Others drank a little at first and then after several weeks their consumption rose to moderate or high levels. Others increased their consumption to much higher levels in two or three weeks. In addition, some rats were spasmodic drinkers—they drank heavily for a day or two and then abstained for several days before indulging again.

Since these rats were on identical diets and their other environmental conditions were the same, the results suggested that heredity was an important factor in determining their individual drinking patterns. A study of other groups of rats, including six closely inbred strains (brother-sister mated for many generations), has given ample evidence to confirm this conclusion. Rats from different sources show wide differences in their drinking patterns and especially in their responses to nutritional supplements. Most inbred strains of animals, however, have not been bred for factors which cause uniformity in alcohol consumption and, hence, there is often wide intrastain variation.

Further experiments confirmed the findings of Mardones and co-workers, as well as those of Brady and Westerfeld, namely, that dietary supplements decrease the alcohol consumption of rats. In fact, we found that the alcohol consumption of practically all the rats in our original colony could be brought to very low levels by administering the known vitamins, including vitamin B₁₂, and linseed oil as a source of unsaturated fat acids, to animals on an otherwise standard diet. On a diet marginal with respect to known B vitamins substantially all the rats would drink alcohol at a high level, but when the deficient nutrients were supplied, alcohol consumption often dropped dramatically overnight and would continue at a low or even zero level thereafter.

When the experiments were shifted to the use of another colony of rats of Wistar origin, exactly similar results were obtained, except that a much larger percentage of rats were "refractory" and continued alcohol consumption even after all known nutrients were supplied in what was thought to be abundant amounts. There was not the slightest doubt, however, about the dramatic response observed in the case of many rats.

It is desirable to point out here that no two laboratories could hope to duplicate each other's detailed findings unless they took extraordinary precautions to use rats of exactly the same genetic origin. Very wide divergences could easily result from strain differences in the animals. It may be well to emphasize, however, that inasmuch as similar results have been obtained hundreds of times, there is not the slightest doubt but that dietary deficiencies in experimental rats bring about greatly increased alcohol consumption, and that meeting these deficiencies (which is not always easy or even possible in all cases)

riboflavin, pantothenic acid or pyridoxin are relatively effective in this respect, but that deficiencies of other nutrients, vitamin A, biotin, and choline are also somewhat effective, as judged both by the number of rats responding and the magnitude of the response. Studies previous to this one had shown that vitamin B₁₂ is certainly a factor and that unsaturated fat acids probably are. It

which indicate that subjecting animals to flashing lights and continuous noise can increase alcohol consumption. The most interesting experiments dealt with 'teetotalers' rats which on stock diets failed to drink alcohol. Some of

psychological stresses bring about physiological changes and it seems reasonable to suppose that the intricate stream of metabolism could be so changed by psychological stress as to augment the needs for specific nutrients. Thus even when psychological factors are involved, the basic change may be metabolic and therefore subject to nutritional influence.

answer is I believe a certain and unequivocal affirmative.

It is needless to say that the gamut of problems which arise in connection with consumption of alcohol—economic, social, health—are not all to be solved by any single stroke. The one problem with which we have been primarily concerned is that of the drinker who cannot control his drinking, the man or woman who is seemingly forced by the first drink to take another and another until he or she becomes incapacitated for useful work. It is our opinion and the findings support it that such craving is essentially physiological and very often beyond intellectual control.

I have come in personal contact with several individuals, all of whom had a terrific craving for alcohol (in some cases of many years' duration) who have been transformed by the use of dietary supplements into individuals who could drink moderately without excess, not for a period of days or weeks but actually for a year or more.²⁰ The first individual was treated about three years ago and as long as he has continued the recommended dietary supplements he has been able to avoid drunkenness and, at the same time, regularly to drink beer, his favorite beverage.

Treatment of alcoholism is complicated by the fact that, for some individuals, the enjoyment derived from drinking is so great that they would as soon part with their right arm as with this pleasure. This is by no means true of all alcoholics. Some would gladly give up alcohol entirely if the physical craving allowed them to do so. People in general have to gain some pleasure from life otherwise they will not want to live and the alcoholic who looks to alcohol

book now in press.²¹ I have presented evidence which shows that even with similar training and similar educational leanings have extremely diverse 'want patterns' and derive their satisfactions from life in entirely different ways. The alcoholic is fortunate if he can cultivate reasons for living which do not include alcohol. For such individuals I believe there is real hope for the future. If nutritional supplements now available will not curb or obliterate

fashion I believe the case is also strong against the existence of individuals who are thoroughly well. Certainly, the great majority of people that I know are unwell in one way or another, whether they consult physicians regularly or

the general population is concerned

In a great many cases, it seems reasonable to suppose, the widespread manifestations of unwellness, lack of vigor, lack of adjustment, failure to mature *etc*, are based upon the fact that each of us \equiv a deviate in a number of different ways. Some of the deviations which we possess may cause trouble and must be understood before they can be managed. Many of the deviations, however, are an inexorable part of life and cannot and should not be dispensed with. Even these desirable deviations, however, need to be understood if we are to understand ourselves.

References

1. " D. T. P. Deane *et al.* 1940 Lancet 1 287
2. " " " " " " " " 31
3. " " " " " " " " 6 506
4. " " " " " " " " 2 Proc Natl Acad Sci 1935
5. " " " " " " " " 210
6. " " " " " " " " 5
7. " " " " " " " " 210
8. " " " " " " " " 5
9. " " " " " " " " 5
10. " " " " " " " " 5
11. " " " " " " " " 5
12. " " " " " " " " 5
13. " " " " " " " " 5
14. " " " " " " " " 5
15. " " " " " " " " 5
16. " " " " " " " " 5
17. " " " " " " " " 5
18. " " " " " " " " 5
19. " " " " " " " " 5
20. " " " " " " " " 5
21. " " " " " " " " 5
22. WILLIAMS R. J. L. J. BERRY & E. BEERSTECHEER, Jr 1950 Texas Repts. Biol Med 8 238
23. RICHTER C. P. 1926 J Exptl Zool 44 397
24. RICHTER C. P. & K. H. CAMPBELL 1940 Science 91 507
25. BRADY R. A. & W. W. WESTERFELD 1947 Quart J Studies Alc 7 499
26. " " " " " " " " 1951 Univ of Studies Alc 13 553
27. " " " " " " " " 1951 Univ of Oklahoma Press. Nor
28. " " " " " " " " 1951 Univ of Oklahoma Press. Nor
29. " " " " " " " " 1951 Univ of Oklahoma Press. Nor
30. " " " " " " " " 1951 Univ of Oklahoma Press. Nor
31. WILLIAMS R. J. 1953 Free and Unequal. Univ of Texas Press Austin, Texas.

Comments on the Paper

DOCTOR DANIEL FELDMAN, New York The evaluation of any treatment regime directed against the disease, chronic alcoholism is fraught with many pitfalls. The peculiar nature of the illness manifests itself in great variations in drinking patterns, and the personality of the chronic alcoholic is such that he may respond with a period of abstinence to almost any procedure used. Unfortunately, these periods invariably end with a resumption of the drinking. I think that, in order to evaluate the effectiveness of any therapy, the patient must elapse before any conclusions can be drawn and a sufficiently long time must elapse before any conclusions can be drawn. I should think that a minimum period of observation of at least a year is necessary before an evaluation of the success of a therapy can be made.

The very important question of inadvertent psychotherapy arises in any critical evaluation of a therapeutic regimen. The part played by such relationships as suggestibility, support, and the magic value of the material used must be honestly assessed. One cannot ignore the fact that because of his personality makeup the alcoholic is positively looking for such psychological aids. As a matter of fact, it is extremely difficult if not virtually impossible for the person dispensing the medication to divorce himself from a psychological relationship of some sort. Obviously this makes it impossible to eliminate this factor in the evaluation of any treatment regime aimed at curing or arresting the disease.

Despite this however, I think that a therapy such as Dr Williams nutritional one can be honestly evaluated if certain things are borne in mind for example the use of the patient's past alcohol pattern as a control. If a significant difference is observed between his drinking pattern before and after the nutritional regime, and if an equal amount of psychological help was given in the past then, one might suspect that the improvement noted might in some way be related to the use of the nutritional substances. I am aware that this statement could be criticized and that it could be pointed out that the individual managing the nutritional therapy might because of his personality supply certain psychological aids that the alcoholic patient did not have before. A controlled experiment making use of identical placebo and carried on for a sufficiently long period of time would be of major importance in determining the part played by nutritional treatment. Because of the great number of variations in drinking patterns and because of the peculiar psychological makeup of the alcoholic, one must be extremely wary in interpreting the results of any particular form of therapy.

As regards Dr Williams report on a patient who became a social drinker following nutritional therapy one can say that it certainly is an unusual result and it would be very important to follow this individual for a long period of time. Not infrequently one sees alcoholics who can drink socially for periods of time but invariably revert to full blown drinking. Another point which is of great interest is Dr Williams deduction that ab-

normal alcohol intake is associated with genetic nutritional abnormalities and, consequently, with nutritional deficiencies. Why should alcohol, a substance which does not as far as we know, correct any of the nutritional deficiencies which may be present be selected by such individuals? The psychological effects of alcohol on the other hand, are well known and if one presumes that the alcoholic chooses this substance for its inhibiting effect on cerebral processes one has at least a logical reason for its use. The emotional disturbances and psychological difficulties of alcoholics are well known and most carefully studied data point to the fact that these difficulties have pre-existed the alcoholism itself. I should like to know Dr. Williams' feelings concerning these psychological difficulties and their relationships to the genetic-nutritional abnormalities which he postulates.

I should like to summarize briefly our experience with Dr. Williams' method of treatment.

A group of 26 patients was treated at the outpatient clinic with nutritional supplements, weekly interviews and in a good number of instances was attending A. A. as well. The study was begun Oct. 20, 1952, but many of the group were not seen until several months later, being added as they appeared at the clinic. Fifteen patients in this group are still being seen with regularity and are not drinking as far as we know. Ten of the group have failed to return and three are known to be drinking heavily. Follow up of the remaining seven has as yet, been unsuccessful. One patient is not attending the clinic because of job interference but reports by telephone. When last heard from he was having no drinking problem and was continuing nutritional therapy. Although there was loss of control this patient would not be considered even a moderately severe alcoholic.

All of the 26 patients studied reported a sense of well being while on the nutritional regimen.

A great deal of psychotherapeutic relationship was present in this group and our experience shows that it is practically impossible to avoid this. If one cuts the patient off short a strong resentment and hurt to the imagined rebuff results and the patient almost invariably drops out. However, inasmuch as most of these patients had failed to control their drinking under similar supportive therapies in the past we were curious to see whether the inclusion of regimen to correct any nutritional deficiencies would make supportive therapy any more successful.

Most of the ten failures occurred in patients who were under treatment longest and it may be that many of the 15 patients still in treatment have not been observed long enough for even the most preliminary of evaluation. It may be significant that the ten failures occurred in so short a period as four months.

Three patients were treated intensively at another clinic. Treatment began in October, 1952, with a potent nutritional supplement and visits were held down to the minimum necessary for accurate observation. Such visits took place at three or four week intervals. The supportive value of such visits became apparent from the patients' own remarks.

One of the patients was a steady pathological drinker whose inebriety was

interrupted by short periods of partial sobriety. He had followed this pattern

so that this patient stated quite happily that the interviews were very helpful, and he would call for additional appointments between his regularly scheduled visits.

The second patient was a severe periodic drinker, going on "benders" once or twice a year. He abstained between bouts. He reported an increased sense of well being during the nutritional regimen. Four months after beginning treatment, he went on a severe prolonged drinking bout, exactly the same as those prior to therapy. For two weeks prior to this, he reported that he could drink an occasional beer.

The third patient is a compulsive periodic drinker with no control. His only sobriety was attained through taking church pledges and, usually, he anxiously awaited the end of such a period and promptly went on a bender. The onset of nutritional therapy coincided with such a pledge. He claims he generally feels better and although he thinks about drinking, there is no compulsion. The pledge has not been in enforcement for two months now and the patient is still not drinking, the first time in his experience that this has happened. Although he feels the "pills" have helped, he states that the fact that he has had a physician at the clinic to whom to come has been a great help and relief to him, and he looks forward to his visits. He has never before had any treatment.

normal alcohol intake is associated with genetic nutritional abnormalities and, consequently, with nutritional deficiencies. Why should alcohol, a substance which does not, as far as we know, correct any of the nutritional deficiencies which may be present, be selected by such individuals? The psychological effects of alcohol on the other hand, are well known and, if one presumes that the alcoholic chooses this substance for its inhibiting effect on cerebral processes, one has at least a logical reason for its use. The emotional disturbances and psychological difficulties of alcoholics are well known and most carefully studied data point to the fact that these difficulties have pre-existed the alcoholism itself. I should like to know Dr Williams' feelings concerning these psychological difficulties and their relationships to the genetic-nutritional abnormalities which he postulates.

I should like to summarize briefly our experience with Dr Williams's method of treatment.

A group of 26 patients was treated at the outpatient clinic with nutritional supplements, weekly interviews and, in a good number of instances, was attending A. A. as well. The study was begun Oct. 20, 1952, but many of the group were not seen until several months later, being added as they appeared at the clinic. Fifteen patients in this group are still being seen with regularity and are not drinking, as far as we know. Ten of the group have failed to return, and three are known to be drinking heavily. Follow up of the remaining seven has as yet, been unsuccessful. One patient is not attending the clinic because of job interference but reports by telephone. When last heard from, he was having no drinking problem and was continuing nutritional therapy. Although there was loss of control, this patient would not be considered even a moderately severe alcoholic.

All of the 26 patients studied reported a sense of well being while on the nutritional regimen.

A great deal of psychotherapeutic relationship was present in this group and our experience shows that it is practically impossible to avoid this. If one cuts the patient off short, a strong resentment and hurt to the imagined rebuff results and the patient almost invariably drops out. However, inasmuch as most of these patients had failed to control their drinking under similar supportive therapies in the past, we were curious to see whether the inclusion of regimen to correct any nutritional deficiencies would make supportive therapy any more successful.

Most of the ten failures occurred in patients who were under treatment longest and it may be that many of the 15 patients still in treatment have not been observed long enough for even the most preliminary of evaluation. It may be significant that the ten failures occurred in so short a period as four months.

Three patients were treated intensively at another clinic. Treatment began in October, 1952, with a potent nutritional supplement and visits were held down to the minimum necessary for accurate observation. Such visits took place at three or four week intervals. The supportive value of such visits became apparent from the patients' own remarks.

One of the patients was a steady pathological drinker, whose inebriety was

and that this patient stated quite frankly that the interviews were very helpful, and he would call for additional appointments between his regularly scheduled visits.

The second patient was a severe periodic drinker, going on "benders" once or twice a year. He abstained between bouts. He reported an increased sense of well being during the nutritional regimen. Four months after beginning treatment he went on a severe prolonged drinking bout, exactly the same as those prior to therapy. For two weeks prior to this, he reported that he could drink an occasional beer.

The third patient is a compulsive periodic drinker with no control. His only sobriety was attained through taking church pledges and, usually, he anxiously awaited the end of such a period and promptly went on a bender. The onset of nutritional therapy coincided with such a pledge. He claims he generally feels better and although he thinks about drinking there is no compulsion. The pledge has not been in enforcement for two months now and the

and any treatment

NUTRITIONAL ASPECTS OF CIRRHOSIS IN ALCOHOLISM—EFFECT OF A PURIFIED DIET SUPPLEMENTED WITH CHOLINE*

By Gerald B. Phillips† and Charles S. Davidson

The Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard) Boston City Hospital, the Department of Medicine Harvard Medical School Boston, Mass. and the National Institute of Arthritis and Metabolic Diseases, U. S. Public Health Service Bethesda Md.

The association of alcoholism and cirrhosis of the liver was observed well over a century ago and is now generally accepted. That alcohol played an etiological role through a direct toxic action on the liver was the natural assumption to be derived from this observation until the demonstration that fatty cirrhosis similar to that seen in man could be produced in animals by means of low protein diets.^{1, 2} Moreover, attempts to produce cirrhosis in animals by the administration of alcohol supplements to an adequate diet have been for the most part unsuccessful.³ As a result, the concept of cirrhosis in the alcoholic has been reoriented toward the nutritional aspects, so much so, in fact, that many consider this disease simply as a nutritional deficiency.

The evidence that cirrhosis in the alcoholic is a manifestation of a nutritional deficiency is not conclusive. To be sure, the reports from various parts of the world which implicate malnutrition unassociated with alcoholism are impressive.^{4, 5} But most of the patients with fatty cirrhosis in this country are chronic alcoholics, and even though dietary intake is usually poor during periods of imbibition alcohol has not been exonerated as an important causative factor, although there is little positive evidence to incriminate it. Another argument for a nutritional basis for the cirrhosis of the alcoholic stems from the therapeutic efficacy of the regimen proposed by Patek and Post,^{6, 7} which emphasizes a nutritious diet. But the success of this regimen in alcoholics drinking up till the time of admission to the hospital in itself cannot be attributed to the resumption of an adequate diet, as withdrawal of alcohol and other influences incident to hospitalization are incurred simultaneously.

Probably the most convincing evidence against an hepatotoxic action of alcohol has been the demonstration that the administration of alcohol in addition to a nutritious diet to chronic alcoholics with liver disease did not prevent hepatic functional and histological improvement in one patient⁸ or exacerbate the liver disease of four patients already treated even though given for as long as 6 to 18 months.⁷ Whether the alcohol consumed by these patients was quantitatively and qualitatively the same as that ingested before hospitalization would not be ascertainable.

Thus although liver disease is certainly related to alcoholism, it is still not clear whether the liver disease of the alcoholic is a result of alcohol poisoning, nutritional deficiency, or both.

The discrepancy in our knowledge of the nutritional aspects of cirrhosis in the laboratory animal as compared to man is comprehensible and is attributable

* This work was done under the sponsorship of the Commission on Liver Disease, Armed Forces Epidemiological Board. It was supported in part by the Office of the Surgeon General, Department of the Army and in part by a grant from Merck and Company, Inc., Rahway, N. J. to Harvard University.

† On assignment from the National Institutes of Arthritis and Metabolic Diseases, U. S. Public Health Service.

largely to the greater limitations of clinical investigation. As a result, we have found it necessary to approach the problem of cirrhosis in the alcoholic in a negative manner. This entails a study of the effects of nutrients on the course of the liver disease and, admittedly, is limited in establishing cause.

In order to determine the effect of a dietary regimen on the course of the liver disease it is almost mandatory to study a patient with active disease, so that definite changes in the hepatic disturbance, in one direction or the other, can be discerned over a relatively short period of time. We have found the most useful indices of activity in the cirrhosis of the alcoholic to be the serum bilirubin and urine urobilinogen concentrations and prefer some elevation of both in the patients to be studied. The criteria that we have generally used to evaluate the course of the liver disease fall into two main categories: (1) "hepatic function" tests, and (2) hepatic histology. Measurement of hepatic size is so inaccurate that the small changes expected in short term studies might easily be missed.

One of the principal difficulties in the evaluation of diets and dietary supplements in chronic alcoholics with active liver disease is the rapidity with which these patients usually improve, in our experience, following hospitalization and consumption of even meager diets. For this reason, many studies dealing with the comparative therapeutic efficacy of diets and dietary supplements in such patients have been relatively unrewarding. Moreover even if a supplementary nutrient does not appear to augment the therapeutic value of a given diet it is nevertheless quite possible that a deficiency of that nutrient was responsible for the liver disease. Rather than compare the therapeutic value of various diets and dietary supplements therefore it is first imperative to dissociate the effects of diet from the effects of alcohol withdrawal and hospitalization on the course of the liver disease, i.e., to determine whether alcohol withdrawal and hospitalization in themselves might ameliorate the liver disease. In this respect the rapid improvement referred to above is advantageous as it permits the short term studies necessitated by the intolerability of markedly deficient diets. If it could be shown that alcoholics with active liver disease did not improve on a basic diet despite alcohol withdrawal and hospitalization but did improve on a more adequate diet, then it could be concluded that one or more constituents of the more adequate diet was responsible for the improvement. Individual dietary factors could then be added to this basic deficient diet to evaluate the effect on the liver disease. Any factor present in food which proves efficacious might seriously be considered to play an important etiological role in the genesis of the liver disease.

This method of approach using deficient diets has been employed in this laboratory for several years. The results of these investigations are reported herewith those published being summarized those unrecorded presented in more detail.

In 1949 Eckhardt, Faloon and Davidson⁸ studied the effects of a protein free diet supplemented with intravenous amino acids on the course of four chronic alcoholics with active cirrhosis. Although the basic diet was essentially devoid of protein (0.1 gm. of nitrogen daily) it was shown to be capable of maintaining nitrogen balance and body weight when 80 gm. of purified casein were added

daily as the sole source of protein¹⁰ It contained no source of the vitamin B complex except choline (30 to 100 mg daily) The four patients were provided with the basic diet plus a multiple vitamin capsule (containing vitamins A C D, B₁, B₂, niacin, B₆, and pantothenic acid) and a 500 to 1000 ml. infusion of a ten per cent solution of amino acids daily The average daily caloric intake ranged from 1700 to 4500 calories While on this regimen for a period of from two to four weeks, all of the patients exhibited a progressive decline in serum bilirubin, two showed a decrease in the size of the liver and spleen and in the one patient who was biopsied serially, there was a decided improvement in hepatic histology In one of the patients, moreover, the liver disease improved despite negative nitrogen balance

In a subsequent study, Eckhardt *et al*¹¹ followed three similar patients for from 8 to 13 days on the same basic diet with the same added vitamins but without the supplementary amino acids Each of the patients showed an improvement in "liver function" tests and a decrease in liver size Liver biopsies performed at the start and finish of the protein free diet period revealed no apparent change in fat content (by histology) except in one patient who received added choline for six days (12 gm of choline chloride daily) These results clearly demonstrate that rapid improvement in the liver disease can occur in alcoholics while they are consuming a diet essentially devoid of protein The possibility arises that this improvement was related either to the alcohol withdrawal and hospitalization, to the protein free diet, or to both It is noteworthy, however that the three patients described had stopped alcohol intake and increased food consumption for from 4 to 7 days before the study began Consequently another explanation for the improvement must be considered *i e*, that these patients may have received enough of one or more beneficial dietary factors during the period just prior to study to initiate and even perpetuate recovery

To obviate this difficulty Phillips Gabuzda and Davidson¹² studied three patients with active cirrhosis who were allegedly drinking up till the time of study which started at the time of admission to the hospital Evidence for this in two of the patients was the presence of Wernicke's syndrome The patients were given no sustenance after admission except for a purified diet consisting solely of solutions of glucose and minerals which was continued for from 8 to 10 days One patient received thiamine, and another thiamine plus multiple vitamins in addition to the diet In contradistinction to the findings of Eckhardt *et al*¹¹ none of these three patients showed significant improvement in hepatic function size or fat content (by histology) After institution of an adequate diet for a similar period, there was improvement in hepatic function in all three patients and a decrease in hepatic fat in two, the third patient not showing a significant diminution until the biopsy taken after two weeks of the diet The failure to improve on a purified diet with subsequent improvement on an adequate diet for a similar period implied that there was something in the adequate diet responsible for the amelioration of the liver disease These results would tend to relegate alcohol withdrawal and hospitalization to subordinate influences in this improvement The failure to improve on this purified diet moreover affords the opportunity to assess the efficacy of individual nu

trients added to this diet on the course of the liver disease. On the basis of the animal studies, choline and methionine would be logical factors to try. Studies of this sort using choline have been carried out and the results follow.

Five chronic alcoholics with evidence of active liver disease, who were drinking heavily and eating poorly until the time of admission to the hospital, were followed on the Thorndike Metabolic Ward. No complicating illnesses were encountered except in one patient (H. C.), who developed transient fever associated with a moderate degree of delirium tremens. After admission measurements the patients received no sustenance other than the purified diet of glucose and saline in four cases and glucose solution only in the one patient (C. W.) with evidence of fluid retention. While on the purified diet, which

400
and
ighly
unpleasant but tolerated it. Three patients had diarrhea (J. M., J. D., C. W.) which stopped within a few days after discontinuing the purified diet. In addition to the purified diet, each patient received choline* orally daily. This supplement was discontinued at the cessation of the purified diet period except in one patient (H. C.), in whom it was continued for 20 days thereafter. One patient (J. D.) received thiamine while on the purified diet because of the diagnosis of Wernicke's syndrome.

Following the purified diet period, a diet containing approximately 1600 calories, 50 gm. of protein and about the same amount of salt and water daily was in the purified diet was instituted in five patients. In three of these

retention, serum bilirubin, thymol turbidity and flocculation, and urine urobilinogen and bile are given in a previous report.¹² Serum alkaline phosphatase† was measured by the method of Bodansky,¹³ serum total cholesterol‡ as described by Schoenheimer and Sperry¹⁴ and by Abell *et al.*¹⁵ and serum phospholipid§ according to Schmidt *et al.*¹⁶ Liver size was estimated by measuring the distance of the liver edge below the right costal margin in the midclavicular line on maximal inspiration. Liver biopsies were performed on admission on the five patients and repeated serially on four. All biopsies were done trans thoracically using the Van Silverman needle and were generally uneventful. For serial biopsies, an attempt was made to insert the needle at the site of the previous puncture.

The results on each patient will be presented separately.

Case Studies

Case 1 Patient J. M. was a 51 year-old white male chronic alcoholic of 30 years duration who was hospitalized 10 to 12 years prior to admission for treatment.

* Choline d hydrogen citrate (Abbott Laboratories, North Chicago, Ill. 60063)

† The authors are grateful to Doctor Stephen Madlock, Surgical Research Laboratory, Boston City Hospital, in whose laboratory the determinations were carried out.

‡ The authors are grateful to Doctor David Horowitz, Chief of Diabetic Clinic, Boston City Hospital, in whose laboratory the determinations were carried out.

daily as the sole source of protein¹⁰ It contained no source of the vitamin B complex except choline (30 to 100 mg daily) The four patients were provided with the basic diet plus a multiple vitamin capsule (containing vitamins A, C, D, B₁, B₂, niacin, B₆, and pantothenic acid) and a 500 to 1000 milliliter infusion of a ten per cent solution of amino acids daily The average daily caloric intake ranged from 1700 to 4500 calories While on this regimen for a period of from two to four weeks, all of the patients exhibited a progressive decline in serum bilirubin, two showed a decrease in the size of the liver and spleen and in the one patient who was biopsied serially, there was a decided improvement in hepatic histology In one of the patients, moreover, the liver disease improved despite negative nitrogen balance

In a subsequent study, Eckhardt *et al*¹¹ followed three similar patients for from 5 to 13 days on the same basic diet with the same added vitamins but without the supplementary amino acids Each of the patients showed an improvement in "liver function" tests and a decrease in liver size Liver biopsies performed at the start and finish of the protein free diet period revealed no apparent change in fat content (by histology), except in one patient who received added choline for six days (12 gm of choline chloride daily) These results clearly demonstrate that rapid improvement in the liver disease can occur in alcoholics while they are consuming a diet essentially devoid of protein The possibility arises that this improvement was related either to the alcohol withdrawal and hospitalization, to the protein free diet, or to both It is noteworthy, however, that the three patients described had stopped alcohol intake and increased food consumption for from 4 to 7 days before the study began Consequently, another explanation for the improvement must be considered, *i e*, that these patients may have received enough of one or more beneficial dietary factors during the period just prior to study to initiate and even perpetuate recovery

To obviate this difficulty, Phillips, Gabuzda, and Davidson¹² studied three patients with active cirrhosis who were allegedly drinking up till the time of study, which started at the time of admission to the hospital Evidence for this in two of the patients was the presence of Wernicke's syndrome The patients were given no sustenance after admission except for a purified diet consisting solely of solutions of glucose and minerals which was continued for from 8 to 10 days One patient received thiamine, and another thiamine plus multiple vitamins in addition to the diet In contradistinction to the findings of Eckhardt *et al*¹¹ none of these three patients showed significant improvement in hepatic function size, or fat content (by histology) After institution of an adequate diet for a similar period, there was improvement in hepatic function in all three patients and a decrease in hepatic fat in two, the third patient not showing a significant diminution until the biopsy taken after two weeks of the diet The failure to improve on a purified diet with subsequent improvement on an adequate diet for a similar period implied that there was something in the adequate diet responsible for the amelioration of the liver disease These results would tend to relegate alcohol withdrawal and hospitalization to subordinate influences in this improvement The failure to improve on this purified diet, moreover, affords the opportunity to assess the efficacy of individual nu

nents added to this diet on the course of the liver disease. On the basis of the animal studies, choline and methionine would be logical factors to try. Studies of this sort using choline have been carried out and the results follow.

Five chronic alcoholics with evidence of active liver disease, who were drinking heavily and eating poorly until the time of admission to the hospital, were followed on the Thorndike Metabolic Ward. No complicating illnesses were encountered except in one patient (H. C.), who developed transient fever associated with a moderate degree of delirium tremens. After admission measurements, the patients received no sustenance other than the purified diet of glucose and saline in four cases and glucose solution only in the one patient (C. W.) with evidence of fluid retention. While on the purified diet, which

was unpleasant but tolerated it. Three patients had diarrhea (J. M., J. D., C. W.) which stopped within a few days after discontinuing the purified diet. In addition to the purified diet, each patient received choline* orally daily. This supplement was discontinued at the cessation of the purified diet period except in one patient (H. C.), in whom it was continued for 20 days thereafter. One patient (J. D.) received thiamine while on the purified diet because of the diagnosis of Wernicke's syndrome.

Following the purified diet period, a diet containing approximately 1600 calories, 80 gm. of protein, and about the same amount of salt and water daily as was in the purified diet, was instituted in five patients. In three of these the caloric and protein contents of the diet were raised to about 3000 calories and 75 gm., respectively, for a subsequent period.

The measurements of hepatic function and size made on admission were repeated at appropriate intervals. The methods for determining bromsulfalein retention, serum bilirubin, thymol turbidity and flocculation, and urine urobilinogen and bile are given in a previous report.¹² Serum alkaline phosphatase† was measured by the method of Bodinsky,¹³ serum total cholesterol‡ is described by Schoenheimer and Sperry¹⁴ and by Abell *et al.*¹⁵ and serum phospholipid§ according to Schmidt *et al.*¹⁶ Liver size was estimated by measuring the distance of the liver edge below the right costal margin in the midclavicular line on maximal inspiration. Liver biopsies were performed on admission on the five patients and repeated serially on four. All biopsies were done trans-thoracically using the Vim Silverman needle and were generally uneventful. For serial biopsies, an attempt was made to insert the needle at the site of the previous puncture.

The results on each patient will be presented separately.

Case Studies

Case 1. Patient J. M. was a 51-year-old white male chronic alcoholic of 30 years duration who was hospitalized 10 to 12 years prior to admission for jaun-

* Choline dihydrogen citrate (Abbott Laboratories, North Chicago, Ill.) only.

† The authors are grateful to Doctor Stephen M. Black, Surgical Research Laboratory, Boston City Hospital, in whose laboratory the determinations were carried out.

‡ The authors are grateful to Doctor David Hurn, in Chief of Diabetic Clinic, Boston City Hospital, in whose laboratory the determinations were carried out.

daily as the sole source of protein¹⁰. It contained no source of the vitamin B complex except choline (30 to 100 mg daily). The four patients were provided with the basic diet plus a multiple vitamin capsule (containing vitamins A, C, D, B₁, B₂, niacin, B₆, and pantothenic acid) and a 500 to 1000 milliliter infusion of a ten per cent solution of amino acids daily. The average daily caloric intake ranged from 1700 to 4500 calories. While on this regimen for a period of from two to four weeks, all of the patients exhibited a progressive decline in serum bilirubin, two showed a decrease in the size of the liver and spleen and in the one patient who was biopsied serially, there was a decided improvement in hepatic histology. In one of the patients, moreover, the liver disease improved despite negative nitrogen balance.

In a subsequent study, Eckhardt *et al*¹¹ followed three similar patients for from 11 to 13 days on the same basic diet with the same added vitamins, but without the supplementary amino acids. Each of the patients showed an improvement in "liver function" tests and a decrease in liver size. Liver biopsies performed at the start and finish of the protein free diet period revealed

improvement in alcoholics while they are consuming a diet essentially devoid of protein. The possibility arises that this improvement was related either to the alcohol withdrawal and hospitalization, to the protein free diet, or to both. It is noteworthy, however, that the three patients described had stopped alcohol intake and increased food consumption for from 4 to 7 days before the study began. Consequently, another explanation for the improvement must be considered, *i.e.*, that these patients may have received enough of one or more beneficial dietary factors during the period just prior to study to initiate and even perpetuate recovery.

To obviate this difficulty, Phillips, Gabuzda, and Davidson¹² studied three patients with active cirrhosis who were allegedly drinking up till the time of study, which started at the time of admission to the hospital. Evidence for this in two of the patients was the presence of Wernicke's syndrome. The patients were given no sustenance after admission except for a purified diet, consisting solely of solutions of glucose and minerals, which was continued for from 8 to 10 days. One patient received thiamine, and another thiamine plus multiple vitamins in addition to the diet. In contradistinction to the findings of Eckhardt *et al*¹¹ none of these three patients showed significant improvement in hepatic function, size, or fat content (by histology). After institution of an adequate diet for a similar period, there was improvement in hepatic function in all three patients and a decrease in hepatic fat in two, the third patient not showing a significant diminution until the biopsy taken after two weeks of the diet. The failure to improve on a purified diet with subsequent improvement on an adequate diet for a similar period implied that there was something in the adequate diet responsible for the amelioration of the liver disease. These results would tend to relegate alcohol withdrawal and hospitalization to subordinate influences in this improvement. The failure to improve on this purified diet, moreover, affords the opportunity to assess the efficacy of individual nu-

TABLE 1

CLINICAL AND LABORATORY DATA OF FIVE CHRONIC ALCOHOLICS WITH CIRRHOSIS OF THE LIVER WHO RECEIVED A PURIFIED DIET AND CHOLINE FOLLOWED BY A MORE ADEQUATE DIET FOR THE SAME PERIOD

All measurements were made at the end of the specified days of diet except the initial measurements which were made on a mixed diet

Patient	J M			C C			J D			C W			H C		
	Intal	Purified diet and choline	1600 cal 50 gm prot diet	Intal	Purified diet and choline	1600 cal 50 gm prot diet	Intal	Purified diet and choline	1600 cal 50 gm prot diet	Intal	Purified diet and choline	1600 cal 50 gm prot diet	Intal	Purified diet and choline	1600 cal 50 gm prot diet and choline
Days of diet	10	10	10	9	9	9	7	7	7	5	5	5	4	4	4
Bromosulfalein % reten	40	21	13	43	34	22	61	60	53	38	39	39	33	34	35
Serum bilirubin } 1 m n	1.5	0.40	0.25	1.3	0.67	0.25	5.2	6.1	3.4	2.5	1.7	1.7	4.5	5.8	6.7
	3.2	1.6	0.4	2.5	1.4	0.74	10.8	11.3	6.8	3.1	3.7	3.7	9.6	12.0	13.8
Thymol turbidity	>87	31	2.9	3.3	3.0	2.6	8.0	6.9	4.4	2.9	2.3	2.3	>87	87	85
Thymol flocculation (D-4+)	0	0	0	1+	0	0	2+	2+	2+	0	0	0	3+	3+	3+
Urine urobilinogen	1.512	1.128	1.32	1.512	1.16	1.32	1.256	1.4	1.4	1.128	1.128	1.128	1.128	1.512	1.128
Urobilinogen (D-4+)	2+	0	0	1+	0	0	2+	3+	2+	3+	±	±	4+	4+	4+
Serum alkaline phosphatase	—	—	—	7.3	5.1	5.8	11.0	8.3	5.3	2.9	3.0	3.3	10.8	6.2	10.4
Serum cholesterol	1049	519	363	184	182	—	3.8	327	327	83	74	75	615	513	501
Serum triglyceride	815	3.8	265	141	149	—	512	316	330	32	35	31	509	531	560
Serum lipase	14	12	11	—	—	—	7	8	9	5	7	6	11	11	11

† See text for quantities

‡ Macleod's unit

§ Bodansky unit

* Below normal for laboratory on mixed diet

dice, anorexia, and weakness, and was told at that time that he had a "very slight cirrhosis." Two years before admission, his alcohol consumption was augmented and food intake concomitantly decreased. For the three weeks before entry, he felt weak and anorectic, ate very little, and decreased his alcohol intake somewhat. On admission, his liver edge was 14 cm below the right costal margin, sharp but not very firm, and only slightly tender. There was no definite edema, ascites, icterus, splenomegaly, or spider telangiectasia.

Results (TABLE 1, FIGURES 1 and 2) During the first ten days of hospitalization, the patient received the purified diet and 6.5 gm of choline dihydrogen citrate orally daily. Over this period, the bromsulfalein retention, serum bilirubin, and urine urobilinogen, which were all abnormal on admission, decreased progressively. The thymol turbidity, which was very high on admission, likewise declined considerably, but since the thymol flocculation was consistently negative, this change may have been related to the steady decrease in serum phospholipid and total cholesterol, which were also quite high on entry. The liver edge was thought to recede 2 cm during this time. Following the ten days of the purified diet, the patient received for the next 30 days the 1600 calorie-50 gm protein diet but no added choline. The improvement in the above measurements continued so that after 30 days of this diet, those remaining abnormal were only a bromsulfalein retention of 7 per cent and a hepatomegaly to 8 cm.

The admission liver biopsy showed a large amount of fat with minimal fibrosis. There were occasional polymorphonuclear leucocytes but no intracellular hyalin was seen. Many of the cells appeared frayed and the nuclei were small and dark. Repeat biopsies done after both 7 and 10 days respectively, of the purified diet and choline disclosed a definite but not marked decrease in fat and many cells with two nuclei, suggesting regeneration. The cells were less frayed and the nuclei were more normal in appearance. A biopsy taken after 10 days of the 1600 calorie 50 gm protein diet revealed still less fat, and one taken after 30 days of this diet showed only a slight to moderate amount of fat, many binucleated cells, and significantly more fibrous tissue than was apparent on the previous biopsies.

Case 2 Patient S. C. was a 48-year old white male chronic alcoholic of 20 years duration who usually drank intermittently in bouts lasting 2 to 3 months and separated by intervals of abstinence lasting 4 to 9 months. He had been drinking steadily and eating little for 8 months prior to admission. During the two weeks before admission, he became quite anorectic and consumed very little food but continued drinking. He claimed that he took 1 to 2 multiple vitamin pills* daily for 1 to 2 months prior to admission. No history of previous hepatic disturbance could be elicited. The admission physical examination revealed facial telangiectases, palmar erythema, and an enlarged liver with a moderately firm tender edge felt about 15 cm below the right costal margin. No definite edema, ascites, icterus, splenomegaly, or spider telangiectasia was made out.

* Unicaps (Upjohn). Each capsule contains Vitamin A 5000 U, Vitamin D 500 U, ascorbic acid 32 mgm, thiamine hydrochloride 2.5 mgm, riboflavin 2.5 mgm, pyridoxine hydrochloride 0.5 mgm, calcium pantothenate 5 mgm, nicotinamide 20 mgm, folic acid 0.25 mgm, and Vitamin B₁₂ activity 1 µgm.

TABLE 1

CLINICAL AND LABORATORY DATA OF FIVE CHRONIC ALCOHOLICS WITH CIRRHOSIS OF THE LIVER WHO RECEIVED A PURIFIED DIET AND CHOLINE FOLLOWED BY A NICOTINOLIC ACID DIET FOR THE SAME PERIOD

All measurements were made at the end of the specified days of diet except the initial measurements which were made on a mission to the hospital

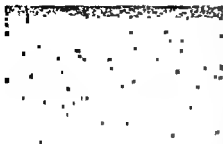
Patient	J M			J D			C W		H C	
	In tal	Pur fed diet and choline	1000 cal 50 gm prot diet	In tal	Pur fed diet and choline	1000 cal 50 gm prot diet	In tal	Pur fed diet and choline	In tal	Pur fed diet and choline
Days diet	10	10	9	7	7	7	5	5	4	4
Irradiation	40	21	13	43	34	22	38	39	33	35
Serum bilirubin	1.5	0.40	0.25	1.3	0.67	0.25	1.7	1.7	4.5	6.7
Thymol turbidity	3.2	1.6	0.74	2.5	1.4	0.74	3.7	3.7	9.6	13.8
Thymol flocculation	>8	3.1	2.9	3.3	3.0	2.6	3.9	2.3	>8.7	8.5
Urine uric nitrogen	0	0	0	1+	0	0	0	0	3+	3+
Serum alkaline phosphatase	1512	1128	132	1512	116	132	1128	1128	1128	1128
Serum cholesterol	2+	0	0	1+	0	0	3+	3+	4+	4+
Serum cholesteryl ester	—	—	—	7.3	5.1	5.8	2.9	3.0	10.8	10.4
Serum triglyceride	1000	519	363	194	182	—	83	74	615	501
Serum albumin	815	378	265	141	149	—	32	35	504	560
Liver size cm	14	12	11	—	—	—	5	7	11	11

† New test for quantitation

‡ McGowan unit

§ Beckman unit

|| Below 100 mg in middle bracket 2 are one mass unit soft ration



third after nine subsequent days of the low calorie (5 gram) diet. The lower (1) day was obtained after twelve subsequent days of the low calorie (5 gram) protein diet. Hematoxylin and eosin stain. X400.

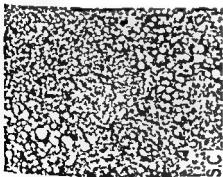
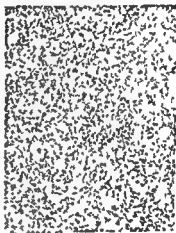
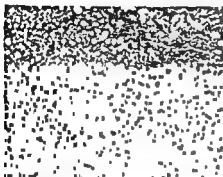


Figure 4. Response for ten weeks. The first biopsy was performed on admission, the second after nine days of the low fat diet and before and the

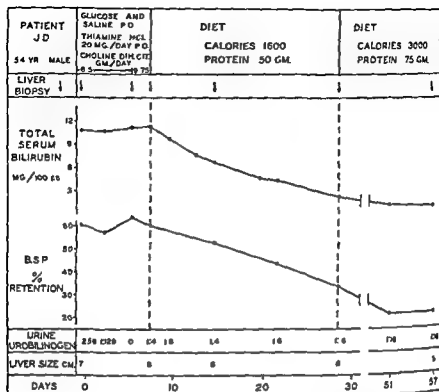


FIGURE 5.

Results (TABLE 1, FIGURE 5) This patient was provided with the purified diet for 7 days. In addition he received 6.5 gm of oral choline dihydrogen citrate daily for the first 5 days, 9.75 gm daily for the last two. 100 mgm of thiamine hydrochloride orally on the first day, and 20 mg orally daily over the next 6 days. On this regimen the serum bilirubin rose slightly but progressively and the bromsulfalein retention remained about the same, although the large amount of dye in the control specimens may have invalidated this measurement to some degree. The urine urobilinogen, however, dropped rather precipitously over this period and, at one time, was thought to be absent. Since stools were all brown this absence could not be attributed to intrahepatic block of bilirubin excretion. It is noteworthy that the patient had profuse watery diarrhea while on the purified diet and conceivable that this disturbance interfered with the production of urobilinogen.

For the next 3 weeks the patient was maintained on a 1600 calorie-50 gm protein diet and for the four subsequent weeks on a 3000 calorie-75 gm protein diet. After two days of the 1600 calorie 50 gm protein diet, the serum bilirubin showed a significant decrease and after 7 days, the serum bilirubin showed a further decline along with a diminution in bromsulfalein retention. These tests continued to improve till the time of discharge. Liver size was difficult

to measure, as the patient's inability to relax frequently prevented detection of

polymorphonuclear leucocytes and no definite intracellular hyalin. The cells appeared frazzled, and their borders were difficult to discern. Many nuclei were small and dark. Repeat specimens obtained after 5 and 7 days respectively of the purified diet and choline displayed essentially the same abnormalities except for a suggestive decrease in fat and less frazzling of the cells. After 7 days of the 1600 calorie-50 gm. protein diet, there was a definite decrease in fat, but no other changes were detected except for an improved cellular architecture and an increase in the number of binucleated cells. The biopsy taken after 3 weeks of this diet had much less fat and intracellular and canalicular bile. Many binucleated cells were observed at this time. The last biopsy specimen, which was secured after 4 weeks of the 3000 calorie 75 gm. protein diet, contained only a minimal amount of fat but considerably more fibrous tissue than on the previous samples.

Case 4 Patient C W. was a 41 year-old white male chronic alcoholic of 25 years' duration who drank in bouts usually lasting a few months each with intervening periods of abstinence of about 3 months. He had been drinking heavily and eating poorly for 7 months prior to admission. He noted ankle swelling and diarrhea for about 5 days and increasing abdominal girth for 3 days before entry. There was no past history of any of the stigmata of liver disease, but he did have ankle swelling 2 years before which disappeared when he stopped drinking. Physical examination on admission disclosed a moderate amount of ascites and ankle edema and a firm smooth nontender liver edge made out with difficulty about 5 cm. below the right costal margin. No icterus, splenomegaly, or spider telangiectasia was found.

he purified diet plus
1600 calorie 50 gm.
protein diet for 16 days.

While he received the purified diet and choline the serum bilirubin rose and the urine urobilinogen remained the same. No initial bromsulfalein test was done. After only one day of the 1600 calorie 50 gm. protein diet the serum bilirubin was significantly reduced and continued to decline over the next 19 days. The bromsulfalein retention and urine urobilinogen, however, showed no change after 5 days of the 1600 calorie 50 gm. protein diet and decreased slightly, if at all, during the remainder of the study. The liver edge was difficult to palpate because of the ascites and the small changes noted are best disregarded.

On admission liver biopsy was done. This showed a moderate amount of fat and marked fibrosis and bile duct proliferation. There were small numbers of polymorphonuclear leucocytes and scattered cells with typical intracellular hyalin.

Case 5 Patient H C. was a 44 year-old white male who drank in spurts for 30 years but steadily for the 2 years before entry. About 4 months prior to admission he increased his alcohol intake and for 2 months prior to admission

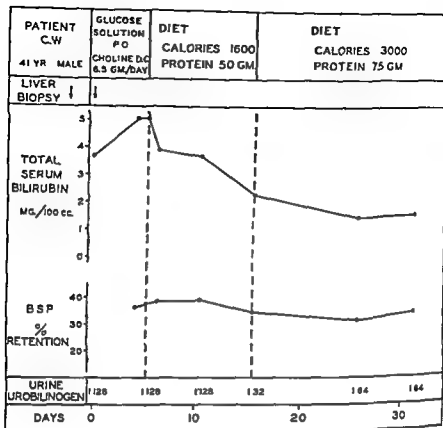


FIGURE 6

he was anorectic and ate very little. He lost approximately 50 pounds in weight over the 3 to 4 months before entering the hospital. Dark urine was noted for 3 months, weakness for 1 month, jaundice for 2 weeks, and light stools for 4 to 5 days before entry. There was no previous history of any manifestations of liver disease. Physical examination at the time of admission revealed a slight icteric tint to the skin with marked scleral icterus, a somewhat tender liver with a firm sharp edge extending 11 cm below the right costal margin, slight gynecomastia, and one spider telangiectasis. No edema, ascites, or splenomegaly was made out.

Results (TABLE 1, FIGURES 7 and 8) This patient was given the purified diet for 4 days, the 1600 calorie 50 gm protein diet for 10 days, the 3000 calorie-75 gm protein diet for 10 days, and the regular hospital diet for 28 days. In addition, he received 3.25 gm of choline dihydrogen citrate daily for the first 24 hospital days. After 4 days of the purified diet and choline, there was a rise in serum bilirubin and urine urobilinogen without a significant change in bromsulfalein retention. After 4 subsequent days on the 1600 calorie 50 gm protein diet and choline, the serum bilirubin rose even further and the bromsulfalein

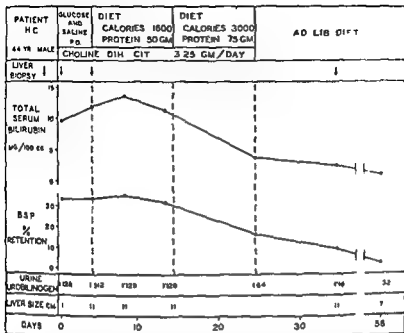


FIGURE 1

retention remained about the same, while the urine urobilinogen dropped. Six days later, however, the former two tests were decreased and, after that, all three tests gradually improved so that they were within normal limits after 52 days in the hospital. The thymol turbidity and flocculation showed no significant change over the first 14 days but were considerably reduced when measured 24 days after entry.

The initial liver biopsy revealed a small amount of fat with a great deal of intracellular and canalicular bile. There were occasional polymorphonuclear leucocytes and probably a minimal amount of intracellular hyalin. Fibrosis was slight in amount. A second biopsy performed after 4 days of the purified diet and choline showed no discernable change except for a possible slight increase in polymorphonuclear leucocytes. The biopsy taken about a month later, however, disclosed no fat but still an abundance of intracellular and canalicular bile. No intracellular hyalin or polymorphonuclear leucocytic infiltration was seen. In contrast to the first two biopsies, there was marked diffuse fibrosis.

Comment

Of five chronic alcoholics who were provided with the purified diet (glucose and saline) and choline orally for from 4 to 111 days, the active cirrhosis seemed

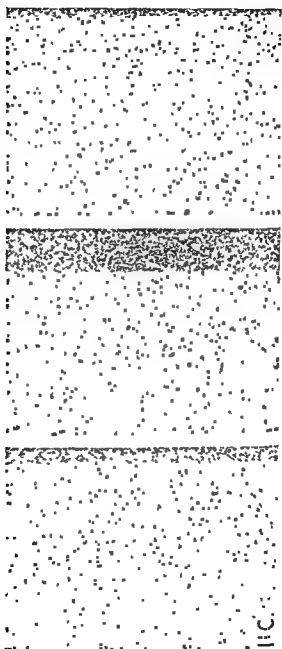


FIGURE 8. Biopsies for patient H. C. The biopsies are in chronological sequence from left to right. The first biopsy was performed on admission; the second, after four days of the purified diet and choline; and the third, after thirty subsequent days of increased dietary fat. Hematoxylin and eosin stain. X250.

to have improved in 2 and worsened in 3. Although it is possible that the latter three patients received a subtherapeutic quantity of choline, two of them consumed as much or more choline per day as did the two patients who improved. Insufficient gastrointestinal absorption may also have occurred since two of the patients whose liver disease became worse had diarrhea. Another factor to consider in one of these three patients was a complicating episode of delirium tremens, which may have contributed to the augmentation of the hepatic disturbance. When these three patients were subsequently provided with a more adequate diet, however, the liver disease improved, two of the patients showing a significant decrease in serum bilirubin within one and two days, respectively, after the diet was instituted. This failure to improve on the purified diet and choline with subsequent improvement on a more adequate diet implies that one or more constituents of the more adequate diet was responsible for the mitigation of the active hepatic disturbance and suggests, in turn, that a deficiency of the same one or more factors was important in the development of the liver disease. These results corroborate the findings in a previous study in which the circumstances were similar except that the purified diet was not supplemented with choline. At least in these three patients, the factor does not appear to be choline alone in the amounts given over these brief periods of study. Neither is the factor just calories, as the more adequate diet was isocaloric with the purified diet. What the therapeutic component of the more adequate diet was, therefore, remains unknown.

Even though three of these patients despite alcohol withdrawal, did not improve until provided with a more adequate diet, the alcohol may still have been an important factor in the development of their liver disease. Its role in this respect might have been to (1) accentuate a nutritional deficiency by

being more deleterious to the hepatic cell conditioned by malnutrition.

Improvement in the active cirrhosis did occur in two of the five patients during the period of the purified diet and choline. That choline may have been the therapeutic agent is suggested by the results of the previous study in which the active cirrhosis of three chronic alcoholics did not improve on the purified diet without choline but did improve rapidly on an adequate diet. Lack of adequate control studies, however, prevents this conclusion, as it is quite possible that the liver disease of the patients in the previous study was related to different etiological factors. Indeed, evidence that more than one important factor may be involved in the treatment and possibly the development of the liver disease of alcoholics is provided in the marked difference of response of the liver disease among the five patients in the present study while on essentially the same regimen.

Qualitative differences in the liver disease of the patients who responded favorably and those who did not may account for this discrepancy in response but are difficult to define. With respect to liver function, the only consistent difference was the higher serum bilirubin in the three patients whose liver function grew worse during the purified diet and choline period. With regard

hepatic histology, which was difficult to assess, the two patients who improved during the purified diet and choline period had a larger amount of hepatic fat than the others. In fact, in the one patient who improved the more dramatically, the marked degree of fatty change was essentially the only hepatic lesion noted. The hepatic fat of these two patients, moreover, appeared to decrease during the purified diet and choline period, but a similar change was also noted in one of the patients whose liver disease simultaneously became worse. Thus, hepatic function did not appear to be related only to the fatty lesion, and other elements of the pathological picture, as fibrosis, intracellular hyaline, and necrosis require consideration. In support of the view that fat is not the only histological lesion to consider was the marked acute hepatic dysfunction present in one patient whose liver biopsy showed only small quantities of fat. It is possible that the response of these patients to various dietary regimens is related to the type of hepatic histological abnormalities present, but the small number of patients in this group forbids any rational attempt at correlation.

One striking feature of the histological studies, which were done serially in four patients, was the apparent increase in fibrosis associated with recovery. Although this change may have resulted from the disappearance of fat and condensation of the remaining tissue, the marked increase in fibrous tissue argued for a genuine progression of the fibrosis. To support this impression was a decided increase in fibrosis seen in one of the patients who initially had only a minimal amount of fat. Although the development of fibrosis could not be related positively to any histological feature, there were two bits of evidence against its relation to fat: (1) The patient with the most hepatic fat developed the least fibrosis, and (2) the patient with minimal fat developed pronounced fibrosis. What gave rise to the fibrosis, if real, therefore, cannot be stated from these studies.

Although not all of the active cirrhosis of chronic alcoholics may be on the same etiological basis, in the great majority of these patients in our experience this active hepatic disease will improve rapidly following hospitalization of the patient and consumption of even meager diets. In the present study, the active cirrhosis improved in two patients who received merely glucose saline and choline and in three patients who were given only 1600 calories and 50 gm of protein daily. From our clinical studies, therefore we believe that effective dietary therapy for the active cirrhosis of the alcoholic is the withdrawal of alcohol and the provision of an adequate diet.

Summary and Conclusions

(1) In a previous study it was shown that the active cirrhosis of three chronic alcoholics failed to improve when these patients were provided with a purified diet (glucose and minerals) but subsequently improved when given an adequate diet for a similar period. These results suggest that one or more constituents of the adequate diet was responsible for this improvement.

(2) In the present study five similar patients were similarly followed except for the addition of choline to the purified diet. In three of the patients choline did not appear to be therapeutic. In the other two patients, choline may have had a beneficial effect.

(3) It is concluded that choline alone in the amounts and for the time given is not therapeutic for the active liver disease of all alcoholics. Whether choline is of definite therapeutic benefit in some cannot be concluded from the data presented.

(4) The marked difference in response of these patients to treatment suggests that the liver disease of the alcoholic may be related to multiple etiological factors.

Acknowledgments

The authors are grateful to Dr G. Kenneth Mallory, the Medical Director of Pathology, Boston City Hospital, for reviewing the histological material.

References

- 1 GILBOY, P. 1949. The nutritional aspects of liver injury. *Med Clin N A* 33: 1657-1669.
- 2 HENSWORTH, H. P. 1950. Lectures on the Liver and its Diseases. Harvard Press, Cambridge, Mass.
- 3 JOLLIFF, N. & F. M. JELLINFA. 1941. Vitamin deficiencies and liver disease in alcoholism. *Proc Soc Exptl Biol Med* 48: 544-547.
- 4 JOLLIFF, N. & F. M. JELLINFA. 1941. Vitamin deficiencies and liver disease in alcoholism. *Quart J Stud Med* 2: 544-547.
- 5 GILBOY, P. 1949. The nutritional aspects of liver injury. *Med Clin N A* 33: 1657-1669.
- 6 PATER, A. T. 1937. Treatment of alcoholic cirrhosis of the liver with high vitamin therapy. *Proc Soc Exptl Biol Med* 37: 329-330.
- 7 PATER, A. T. 1937. Treatment of alcoholic cirrhosis of the liver with high vitamin therapy. *Proc Soc Exptl Biol Med* 37: 329-330.
- 8 PATER, A. T. 1937. Treatment of alcoholic cirrhosis of the liver with high vitamin therapy. *Proc Soc Exptl Biol Med* 37: 329-330.
- 9 PATER, A. T. 1937. Treatment of alcoholic cirrhosis of the liver with high vitamin therapy. *Proc Soc Exptl Biol Med* 37: 329-330.
- 10 PATER, A. T. 1937. Treatment of alcoholic cirrhosis of the liver with high vitamin therapy. *Proc Soc Exptl Biol Med* 37: 329-330.
- 11 PATER, A. T. 1937. Treatment of alcoholic cirrhosis of the liver with high vitamin therapy. *Proc Soc Exptl Biol Med* 37: 329-330.
- 12 PATER, A. T. 1937. Treatment of alcoholic cirrhosis of the liver with high vitamin therapy. *Proc Soc Exptl Biol Med* 37: 329-330.
- 13 PATER, A. T. 1937. Treatment of alcoholic cirrhosis of the liver with high vitamin therapy. *Proc Soc Exptl Biol Med* 37: 329-330.
- 14 PATER, A. T. 1937. Treatment of alcoholic cirrhosis of the liver with high vitamin therapy. *Proc Soc Exptl Biol Med* 37: 329-330.
- 15 PATER, A. T. 1937. Treatment of alcoholic cirrhosis of the liver with high vitamin therapy. *Proc Soc Exptl Biol Med* 37: 329-330.
- 16 PATER, A. T. 1937. Treatment of alcoholic cirrhosis of the liver with high vitamin therapy. *Proc Soc Exptl Biol Med* 37: 329-330.
- 17 PATER, A. T. 1937. Treatment of alcoholic cirrhosis of the liver with high vitamin therapy. *Proc Soc Exptl Biol Med* 37: 329-330.
- 18 PATER, A. T. 1937. Treatment of alcoholic cirrhosis of the liver with high vitamin therapy. *Proc Soc Exptl Biol Med* 37: 329-330.
- 19 PATER, A. T. 1937. Treatment of alcoholic cirrhosis of the liver with high vitamin therapy. *Proc Soc Exptl Biol Med* 37: 329-330.
- 20 PATER, A. T. 1937. Treatment of alcoholic cirrhosis of the liver with high vitamin therapy. *Proc Soc Exptl Biol Med* 37: 329-330.

produced by feeding alcohol or sugar and its prevention by choline Brit Med J 2
1001 1006

- 18 KLATSKIN G, H M GEWIN, & W A KREHL. 1951 Effects of prolonged alcohol ingestion on the liver of the rat under conditions of controlled adequate dietary intake Yale J Biol and Med 23 317-331

Discussion of the Paper

DOCTOR HAROLD BLUMBERG, ENDO PRODUCTS INC, NEW YORK This investigation by Doctor Davidson and Doctor Phillips is certainly very interesting and valuable Theoretically, of course, the value of choline in alcoholic fatty cirrhosis might be tested better perhaps by a somewhat different type of experiment although it is appreciated that this might be difficult to do in the clinic Choline by itself, or with glucose, cannot be expected to constitute complete therapy In addition to removal of excess fat there must be regeneration of hepatic parenchyma, and the latter requires protein Choline cannot provide this necessary protein It has been suggested only as an adjuvant in the high protein therapy of cirrhosis There should be two groups of patients both receiving an adequate amount of protein or amino acids, but not unusually high in methionine One of the groups should, in addition, receive choline and the possible value of choline should be gauged by the rapidity and extent of the improvement as compared with the noncholine group

In 1944 Doctor Russakoff and I reported a partial approach toward this method Included in a small series of cirrhotic patients with ascites were three who showed no significant improvement after two weeks on a complete diet adequate in protein (protein, 125-200 gm, carbohydrate, 250-300 gm, fat, 50-60 gm) Choline chloride was then added to the regimen in dosages of 3-6 gm per day Marked clinical improvement, with decrease in ascites occurred in all three patients within a week following the start of choline therapy The coincidence of clinical improvement and choline administration suggested that choline might have therapeutic value

In our early experiments on fatty cirrhosis in rats it appeared that the most extensive fibrosis was observed in animals that, after a long period on a high fat and low protein diet, were given additional protein in alternating periods in order to permit some recovery of weight and to prolong survival Incidentally the alternation of injury and repair may be somewhat analogous to the pathological history of alcoholic cirrhosis in humans This was the reason for my previous suggestion that, after this type of liver injury, the high protein therapy not only produces regeneration of normal liver cells, but also may promote the full development of the incipient fibrosis in the areas where the cells have already been badly damaged Therefore, it is interesting in this connection, that Doctor Davidson and Doctor Phillips have found that, with complete dietary therapy, their patients showed not only clinical improvement and decreased liver fat but also an increase in fibrosis in the liver biopsies

THE PATHOLOGY OF DIETARY LIVER NECROSIS—A PRELIMINARY REPORT

By George L. Fite

U. S. Public Health Service, National Institutes of Health, Bethesda Md

Hepatic necrosis induced by dietary means, although originally observed in

well established, with deficient protein amino-acid fractions of the diet the common denominator of the several dietary means employed

The necrosis is, in the last analysis, the end result of an underlying degenerative process. Schwarz⁴ has introduced the term *dietary necrotic liver degeneration* to emphasize the degeneration as the primary phenomenon. Although much information has been gained with regard to substances which will prevent the necrosis from occurring, still the basic nature of the degenerative process behind it is as yet little understood. In various reports, necrosis has been observed anywhere from the 30th to the 90th day or later following the placing of animals on a suitable dietary regime, with death of the animals usually follow-

method of Schwarz was the provision of a yeast providing the protein

This diet has been regarded as being primarily deficient in respect to vitamin E and "Factor 3". From the standpoint of the suitability for histologic study of livers of animals administered this diet, there are certain great advantages, which stem from the consistently high percentage (90-100 per cent) of animals dying with liver necrosis during the span of the 35th to the 50th day, providing many early lesions.

In comparing in a general way the lesions of these animals with those described by other writers, using other but not entirely different diets, it is possible to make the general statement that the lesions are in every way so similar as to indicate that the problem, the nature of the underlying degeneration is the same. Differences are those of degree and time, but not of general character.

In addition to changes in the liver in the animals studied, other changes especially in the kidney and in the fore stomach have been seen, the latter being of the character known to occur in vitamin F deficiency. Although not considered here in detail, they are added evidence of the deficient quality of the diet used.

Livers of more than 400 animals drawn from the experiments of Schwarz have been studied, presenting a broad view of the field. The problem is complex, and the picture is complex. This incomplete report indicates certain lines of investigation of promise for the future and cautions against unwarranted inferences. In general, findings are in harmony with those of other investigators. Interpretations may differ.

produced by feeding alcohol or sugar and its prevention by choline Bnt Med J 2
1001-1006

- 18 KLATSKIN, G., H. M. GEWIN, & W. A. KREHL. 1951. Effects of prolonged alcohol ingestion on the liver of the rat under conditions of controlled adequate dietary intake. Yale J Biol and Med 56: 317-331.

Discussion of the Paper

DOCTOR HAROLD BLUMBERG, ENDO PRODUCTS INC., NEW YORK. This investigation by Doctor Davidson and Doctor Phillips is certainly very interesting and valuable. Theoretically, of course, the value of choline in alcoholic fatty cirrhosis might be tested better perhaps by a somewhat different type of experiment, although it is appreciated that this might be difficult to do in the clinic. Choline by itself, or with glucose, cannot be expected to constitute complete therapy. In addition to removing hepatic parenchyma, and the lack of this necessary protein. It has protein therapy of cirrhosis. receiving an adequate amount of protein or amino acids, but not unusually high in methionine. One of the groups should, in addition, receive choline and the possible value of choline should be gauged by the rapidity and extent of the improvement as compared with the noncholine group.

In 1944 Doctor Russakoff and I reported a partial approach toward this method. Included in a small series of cirrhotic patients with ascites were three who showed no significant improvement after two weeks on a complete diet adequate in protein (protein, 125-200 gm, carbohydrate, 250-300 gm, fat, 50-60 gm). Choline chloride was then added to the regimen in dosages of 3-6 gm per day. Marked clinical improvement, with decrease in ascites occurred in all three patients within a week following the start of choline therapy. The coincidence of clinical improvement and choline administration suggested that choline might have therapeutic value.

In our early experiments on fatty cirrhosis in rats it appeared that the most extensive fibrosis was observed in animals that, after a long period on a high fat and low protein diet, were given additional protein in alternating periods in order to permit some recovery of weight and to prolong survival. Incidentally, the alternation of injury and repair may be somewhat analogous to the pathological history of alcoholic cirrhosis in humans. This was the reason for my previous suggestion that, after this type of liver injury, the high protein therapy not only produces regeneration of normal liver cells, but also may promote the full development of the incipient fibrosis in the areas where the cells have already been badly damaged. Therefore, it is interesting in this connection that Doctor Davidson and Doctor Phillips have found that, with complete dietary therapy, their patients showed not only clinical improvement and decreased liver fat but also an increase in fibrosis in the liver biopsies.

THE PATHOLOGY OF DIETARY LIVER NECROSIS—A PRELIMINARY REPORT

By George L. Fite

U. S. Public Health Service, National Institutes of Health, Bethesda, Md.

Hepatic necrosis induced by dietary means, although originally observed in work designed to throw light on cirrhotic processes, has become recognized as a

common denominator of the several dietary means employed^{1, 2, 3, 4, 5}

The necrosis is, in the last analysis, the end result of an underlying degenerative process. Schwarz⁶ has introduced the term *dietary necrotic liver degeneration* to emphasize the degeneration as the primary phenomenon. Although much information has been gained with regard to substances which will prevent the necrosis from occurring, still the basic nature of the degenerative process behind it is as yet little understood. In various reports, necrosis has been observed anywhere from the 30th to the 90th day or later following the placing of animals on a suitable dietary regime, with death of the animals usually following the necrosis promptly.

In the current investigations, the "yeast" method of Schwarz was the provocative dietary procedure, a special type of *Torula* yeast providing the protein in a diet complete in most other respects. This diet has been regarded as being primarily deficient in respect to vitamin E and "Factor 3". From the standpoint of the suitability for histologic study of livers of animals administered this diet, there are certain great advantages which stem from the consistently high percentage (90-100 per cent) of animals dying with liver necrosis during the span of the 35th to the 50th day, providing many early lesions.

In comparing in a general way the lesions of these animals with those described by other writers, using other but not entirely different diets, it is possible to make the general statement that the lesions are in every way so similar as to indicate that the problem, the nature of the underlying degeneration is the same. Differences are those of degree and time, but not of general character.

In addition to changes in the liver in the animals studied, other changes especially in the kidney and in the fore stomach have been seen, the latter being of the character known to occur in vitamin E deficiency. Although not considered here in detail, they are added evidence of the deficient quality of the diet used.

Livers of more than 400 animals drawn from the experiments of Schwarz have been studied, presenting a broad view of the field. The problem is complex, and the picture is complex. This incomplete report indicates certain lines of investigation of promise for the future, and cautions against unwarranted inferences. In general, findings are in harmony with those of other investigators. Interpretations may differ.

produced by feeding alcohol or sugar and its prevention by choline Brit Med J 2
1001-1006

- 18 KLATSKIN G H M GEWIN & W A KREHL 1951 Effects of prolonged alcohol
ingest on the liver of the rat under conditions of controlled adequate dietary intake
Yale J Biol and Med 23 317-331

Discussion of the Paper

DOCTOR HAROLD BLUMBERG, ENDO PRODUCTS INC, NEW YORK This investigation by Doctor Davidson and Doctor Phillips is certainly very interesting and valuable. Theoretically, of course the value of choline in alcoholic fatty

therapy. In addition to removal of hepatic parenchyma and the latter this necessary protein. It has been protein therapy of cirrhosis. There should be two groups of patients receiving an adequate amount of protein or amino acids, but not unusually high in methionine. One of the groups should, in addition receive choline and the possible value of choline should be gauged by the rapidity and extent of the improvement as compared with the noncholine group.

In 1944 Doctor Russakoff and I reported a partial approach toward this method. Included in a small series of cirrhotic patients with ascites were three who showed improvement after two weeks on a complete diet adequate in protein, carbohydrate, 250-300 gm fat to the regimen in dosages of 50-60 gm 3-6 gm per day. Marked clinical improvement, with decrease in ascites occurred in all three patients within a week following the start of choline therapy. The coincidence of clinical improvement and choline administration suggested that choline might have therapeutic value.

In our early experiments on fatty cirrhosis in rats it appeared that the most extensive fibrosis was observed in animals that, after a long period on a high fat and low protein diet, were given additional protein in alternating periods in order to permit some recovery of weight and to prolong survival. Incidentally the alternation of injury and repair may be somewhat analogous to the pathological history of alcoholic cirrhosis in humans. This was the reason for my previous suggestion that after this type of liver injury, the high protein therapy not only produces regeneration of normal liver cells, but also may promote the full development of the incipient fibrosis in the areas where the cells have already been badly damaged. Therefore it is interesting in this connection that Doctor Davidson and Doctor Phillips have found that with complete dietary therapy their patients showed not only clinical improvement and decreased liver fat but also an increase in fibrosis in the liver biopsies.

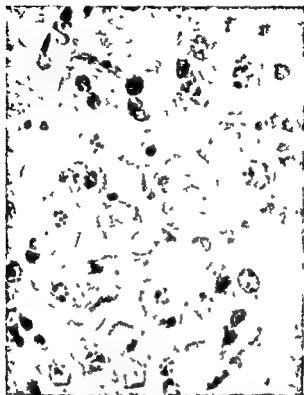


FIGURE 1. Diffuse karyolysis and karyorrhexis of liver cell nuclei during a *u* phase

may often appear normal or nearly so. In glycogen and fat stained sections it is possible to find many cells containing glycogen but no fat yet illustrating the beginnings of the karyolysis. However it is clear that these cells do lose their glycogen in a short time probably a matter of a few hours once the process is started and likewise acquire cytoplasm lipoids at the same time. Once the karyolysis is well established the cytoplasm often appears frayed out some what watery perhaps increased in overall volume. But the point to be made is that the nuclear degeneration often appears to precede the cytoplasmic.

It is dangerous to draw too much inference from this histologic change. The liver may appear normal on the 33rd day and necrotic on the 34th. It is obvious that the histologic evidence of liver cell injury is only a partial measure of what goes on.

Another curious finding is the occurrence of numerous mitotic figures in liver cell nuclei in occasional animals which are undergoing this degenerative process. The mitoses occur at random anywhere and are far less common than seen in livers undergoing regeneration. Evidently they are as though the primitive mechanism of repair

An experiment was undertaken in which animals were killed at predetermined intervals after being on the basic diet 24, 27, 30, and 34 days a total of 30 animals being used. Frank necrosis was seen in two animals killed on the 34th day. All livers were sectioned and studied for fat and glycogen.

The results paralleled those of Abell, Beveridge, and Fisher,⁷ who employed similar procedures. The glycogen was normal in all animals except four killed on the 34th day. The two which showed early necrosis showed no glycogen. In the two others, it was diminished, being found largely in portal areas. There were no definite abnormal fat deposits except in the two animals with early necrosis, in which droplets of moderate size were distributed in moderate numbers throughout the liver. The progressive increase in fat seen by Abell⁷ was not observed in the shorter elapsed time of this experiment.

Perhaps the most significant finding of this experiment is the essentially normal histologic appearance of the liver almost up to the point of appearance of the necrosis. It seems important to emphasize the fact that although the deficiency will routinely kill animals at about the 45th day, there is little histologic alteration in evidence almost up to the point at which necrosis appears.

Types of Liver Cell Degeneration

In the animals dying with or without frank necrosis, two distinctive types of changes in the liver cells had been observed.

The first of these is diffuse karyolysis and karyorrhexis of the liver cell nuclei with relatively little change in the cytoplasm of the liver cells (FIGURE 1).

whether, in these animals the deficiency affects primarily the nucleus of cells, or whether one is dealing with a deficiency in substances essential to formation of nuclear protein or essential to nuclear metabolism. The strong impression is that the injury to the liver cell in these animals is a very different thing from the injury due to carbon tetrachloride or other chemical substance in which the cytoplasm of the cell must necessarily absorb quantities of the chemical irritant.

The sequence of the karyorrhexis-karyolysis process appears to be roughly as follows:

- (1) A rearrangement of the nuclear chromatin.
- (2) A diminution in size of the nucleus,
- (3) Dissolution of the nuclear capsule,
- (4) Contraction of the volume of the nucleus as a whole, yet with outlines preserved.

Following dissolution of the nuclear membrane, the nuclear contents do not fuse with the cytoplasm but the nuclear form is retained for a considerable period of time. Many nuclei will also shrink to become pyknotic, but this type of degeneration appears to follow later.

In cells showing the karyolytic process in its earliest phase, the cytoplasm

killed in both early and subacute stages of the process, as well as in a few animals which had been fed protective substances after the period of potential necrosis was well under way. It seems probable that the eosinophilic granules of the cytoplasm become calcified only infrequently, the determining factors being unknown.

Up to the present time, the exact relation of this type of degenerative process to the other is not understood. This type of lesion had not been seen too frequently to the point of calcification of the bodies, but it has been possible to demonstrate the early cytoplasmic change in the liver cell many times. The mechanism appears to be quite different from that of diffuse karyolysis. Here the nucleus is unaltered until after the process of granular body formation is well developed. That the granules may develop from altered mitochondria is a strong possibility, proof of which is as yet wanting.

The Circulatory Factors

Himsworth and his associates^{3, 4} have stressed the circulatory factor in liver necrosis, especially with respect to the distribution of lesions. The distribution of the areas of necrosis in these animals has been essentially the same as that observed by all other investigators: more common at the free margins of the lobes, more common where these are in contact with other viscera, more common in lobes which are thin, than thick. Himsworth went to great lengths to show that a failure of splenic and portal blood to mix promptly might be responsible for differences, in other words, that the differences might be related to a condition when one lobe receives more blood directly from the intestines, while another receives a majority of its venous blood from the spleen. While not attempting to repeat all of Himsworth's detailed studies relative to this problem, India ink was injected into some thirty animals on the 33rd day, at a time when liver necrosis was incipient. The injections were made in the spleen, and the animals killed a few hours later. As nearly as could be judged from gross observation of blackening of the liver immediately following the injection, and from microscopic sections, the ink was evenly distributed to all lobes, and promptly taken up by the Kupffer cells. Only minor differences of lobular distribution were observable, such as might be attributed to a temporary increase or decrease of flow to a given area. Several animals showed early stages of degeneration, in which the ink was deposited as in normal areas. Three animals with early necrosis showed absence of ink from some necrotic areas, but by no means all. The only conclusion to be drawn is that any alteration in circulation occurs after and not before, the occurrence of necrosis.

A second consideration is the distribution of necrotic areas with respect to the liver lobule. Experience has been that there is no absolute rule with respect to the occurrence of areas of degeneration or necrosis, although there are preponderances.

(1) There is no focal necrosis. Areas of necrosis are invariably larger than the individual lobule.

(2) The usual area of frank necrosis involves an area of the liver without respect to its main blood supply. Exceptions occur, however, in which the

against destruction has been evoked. Although this phenomenon has been seen in only about 2 per cent of all the livers examined, it is too striking to go without mention, and is further evidence of the profundity of the alterations in the liver cell.

A second type of degenerative change has been observed in many animals, often in those showing varying degrees of diffuse karyolysis, but not conjointly within the same cell. This is seen first as fine refractile eosinophilic granules evenly distributed in the cytoplasm. These are quite small, of the order of $1-2\ \mu$ in diameter. When cells containing these bodies become separated from neighboring cells, as they often do, the granules become larger, and finally undergo calcification (FIGURE 2).

The various stages of formation can be followed through various animals, although in a given animal the changes throughout are essentially of the same age and degree. The cell form is often retained, perhaps supported by the calcific bodies. The nucleus is unaltered at first, later fading out of existence without undergoing karyorrhectic changes.

This sequence of events has been seen to best advantage in animals dying or



FIGURE 2. Cytoplasmic granules in degenerating liver cells, advanced stage with calcification.

Once necrosis has occurred, the resulting effects on the circulation in the

anoxemia may produce still further change. In most cases the animal dies quickly for this to be histologically clear-cut.

A second result of the necrosis is the 'hemorrhage' which is seen frequently. For the most part this so-called hemorrhage is an accumulation of blood in sinusoids at the margins of necrotic areas rather than extravasation out of the normal channels. Another type, however, consists of pools around afferent veins suggesting some degree of back tracking of blood from efferent veins in necrotic areas. Again these accumulations are within sinusoids and not hemorrhage (i.e. the escape of blood from vascular pathways) probably do not occur.

It is impossible to estimate the degree to which simple congestion can operate to provoke frank necrosis. Yet when one sees frequently the degeneration which has occurred in more 'normal' areas in the necrotic livers it is easily supposed that an extremely slight alteration in blood flow might operate to initiate or to advance the formation of a large area of necrosis. Under the circumstances it hardly seems necessary to conceive a gross alteration or variation in the hepatic circulation to explain the change from degeneration to necrosis.

Delayed Liver Necrosis

On a certain number of the animals have actually died from necrosis. Here the picture may be different in several ways from that of animals dying early in the disease.

(1) The picture of diffuse karyolysis is shown to better advantage or to a greater degree in animals dying at about the 50th day than in animals dying on the 30th day on the basic diet. This seems to support the idea that the diffuse

surviving longer periods of time seem to illustrate degenerative processes to better advantage than animals dying in the earlier acute phase.

Abell² speaks of submassive and recurrent submassive necrosis. These changes are similar to those we have observed in some animals dying at later time intervals. He illustrates the phenomenon of numerous mitotic figures in this 'recurrent submassive' variety. In our animals histologic evidence of recurrent necrosis has been seen (1) where a delicate balance between death and survival was maintained, or (2) when scars and regenerated areas obviously resulted.

h scars in

means at



FIGURE 3 Gross appearance of liver of rat dying with a uterus ossified

necrotic areas have an irregular ramifying serpentine pattern analogous to the branchings of *efferent veins*

(3) There is commonly a narrow zone of preserved cells adjacent to portal areas. In frankly necrotic areas this is extremely narrow at best. In degenerating areas a layer 3 to 5 cells thick around portal zones may be normal.

(4) Inversely there are many examples in which the preponderant areas of degeneration or necrosis or both are in a sense centrolobular. There is never however a discrete central zone but a broad area making up most of the lobule.

It becomes clear that the circulatory arrangement of the liver does have an effect on the distribution of the areas of necrosis in that the more immediately nourished portal cells suffer the degenerative changes slightly less.

DIETARY METHODS FOR INDUCTION OF NECROTIC LIVER DEGENERATION

By Marianne Goettsch

University of Puerto Rico School of Medicine, San Juan, Puerto Rico

Necrotic liver degeneration is readily induced in young rats by the dietary control of two factors, sulfur containing amino acids and Vitamin E. I am aware that the modern interpretation requires at least three factors: cystine, vitamin E and Factor 3, but I shall leave their elucidation to Doctor Klaus Schwarz. Doctor Daft has given an interesting account of the historical sequence of factors in nutritional liver injuries. The preventive effect of vitamin

recognized the importance, in experiments of this type, of restricting the

(1951), which was the protein used by the original investigators. The composition of the diets is given in TABLE 1. Diet 1 is typical of diets used in Vitamin E experiments. Diet 2 contains the same ingredients as the low protein diets 3 to 6. When α -tocopherol was added, it met the minimum protein requirement for growth and reproduction in several generations of rats.

Supplements were introduced at the following concentrations: DL methionine, 0.1 to 0.3 per cent, L-cystine, 0.1 to 0.3 per cent, and dl- α -tocopherol acetate, 2 mgm per rat per week.

Procedure. Wistar or Sprague Dawley rats were used. Breeding rats were cared and bred on the low vitamin E but adequate diets 1 and 2 which were equally suitable for the preparation of young. To permit the birth of young the rats were given 10 mgm of α -tocopherol at the beginning of the first gestation, 20 mgm at the second and 30 mgm at the third. The young did not develop muscular dystrophy at the end of lactation. In this way young with minimal low stores of vitamin E were obtained.

These young rats were given diets 3 to 6 from the 21st day at which time the body weight was approximately 35 gm.

Results and Discussion

Rats with the low casein diets grew poorly. Nearly all of them (about 90 per cent) died suddenly after 5-40 days. In recent trials the incidence reached 100 per cent and none of the rats survived for longer than 20 days. Liver necrotic degeneration was observed grossly and confirmed by Doctors Koppisch and Izquierdo of the Department of Pathology of the University of Puerto Rico School of Medicine by microscopic examination. Some of the rats presented early lesions of muscular dystrophy.

any time prior to its actual occurrence. In a number of animals so protected, and killed later, the appearance of the liver has usually been that of the normal animal. In a few, scarring and regeneration have appeared. Except for these evidences of repair and regeneration of frankly necrotic areas, no changes have been seen. Thus present indications are that the degenerative changes are reversible to a substantial degree although the limits have yet to be defined.

Cirrhosis has never been observed in these animals on the necrogenic diet nor has the picture of the fatty liver of choline deficiency been seen.

References

1. *Physiol* 25 363
2. 1942 *Proc Soc Exptl Biol Med* 50 1
3. *In Sci* 5 93
4. UBERGER 1945 *Brit J Exptl Path* 25
5. SCHWARZ K. 1944 *Z physiol Chem* 281 101
6. SCHWARZ K. 1951 *Proc Soc Exptl Biol Med* 77 818
7. ABELL M R., J M R BEVERIDGE, & J H FISCHER 1950 *Arch Path* 50 1

Discussion of the Paper

DOCTOR HANS POPPER, *Hektoen Institute, Chicago, Ill* I should like to congratulate Doctor Fite on a thorough and much needed investigation upon the histologic changes in acute hepatic necrosis. It is worth emphasizing that histologically insignificant changes precede the explosive sudden breakdown of the liver cells and that nuclear changes usher in the lesion. The eosinophilic necrosis described is also interesting from the point of view of the human

tions granular coagulation necrosis of the cytoplasm of the character of Mallory bodies is more common. This raises the question as to the general behavior of the cytoplasmic basophilia supposedly due to the presence of pentose nucleic acid in nutritional necrosis. I also should like to ask about possible alterations of the reticulum framework in the animals studied. In most instances of human hepatic necrosis presumably produced by either viruses or chemical toxins it remains intact whereas it is broken in some types of toxic hepatitis and as the result of ischemia. Obviously, the reticulum changes would be of great importance in recovery stages from such necrosis.

low E—high casein diet until they weighed 70–100 gm. With increasing initial body weight, there was a decrease in the incidence of liver necrotic degeneration and an increase in the time of survival. This observation confirms that of Lundan and Himsforth (1950).

Initial stores of vitamin E in the rat. There is great variation among rats in their ability to store vitamin E in the tissues and in the requirement of vitamin E for reproduction and for the prevention of muscular dystrophy. In vitamin E low rats that were given one single dose of 2 mgm α tocopherol on the 10th day of lactation, the incidence of liver necrotic degeneration was reduced 50 per cent.

The discordant results among different laboratories in the induction of liver degeneration presumably have been associated with the amount of α tocopherol supplied during the pre experimental period. It was recently observed that some rats reared and maintained with a commercial ration, underwent resorption gestations when they were given the low E—high protein diet from the beginning of gestation. These results suggest an explanation for the induction of liver necrotic degeneration in rats that have not been purposefully prepared to contain low initial stores of vitamin E.

Addition of methionine and cystine. The preventive effect (Weichselbaum 1935; Daft, Sebrell, and Lilhe, 1942) of the sulfur containing amino acids is well known. Although these experiments were not quantitative as were those of Schwarz (1952), the addition of 0.3 per cent of either DL-methionine or L-cystine to the low casein diets prevented the liver necrosis in a high proportion of the rats under the given conditions. As with other groups of rats chronic muscular dystrophy and sterility developed in all rats that did not

Effect of α tocopherol. Liver necrotic degeneration was prevented in all rats receiving 2 mgm α tocopherol per week, in confirmation of the results of Schwarz (1944), Gyorgy (1947) and Gyorgy and Goldblatt (1949). The α tocopherol not only prevented liver necrotic degeneration but also protected the rats from muscular dystrophy and sterility. When the rats were sacrificed after a 50-day period, the following results were obtained:

Thus all	
livers are	
injury was highest among rats with the high lard diets.	The addition of 0.3
per cent fat	and cirrhosis
	the lesions
	er and Eck

(1951) Earle and Victor (1941), and others

Summary

(1) With low E—low-crude casein diets liver necrotic degeneration appears especially in young rats deprived of vitamin E. Early lesions of muscular dystrophy may occur.

TABLE I
COMPOSITION OF EXPERIMENTAL VITAMIN E-LOW DIETS

Constituents*	Diets for breeding rats†		Isocaloric low protein diets			
	1	2	3	4	5	6
Crude casein	26.6	20.0	7.5	8.3	10.0	11.4
Yeast brewers' type	16.6	—	—	—	—	—
Cornstarch	33.3	68.0	86.5	79.7	64.0	48.0
Salt mixture‡	3.4	4.0	4.0	4.0	4.0	4.6
Lard	18.3	6.0	—	6.0	20.0	34.0
Cod liver oil	1.8	2.0	2.0	2.0	2.0	2.0

* Sum of constituents = 100.0 g.

After some weeks, the survivors ceased to grow and acquired the chronic muscular dystrophy that was described by Einarson and Ringsted (1938) in low E rats. A few that survived 90 days were bred. They manifested the typical sterility of vitamin E deprivation (Evans and Bishop, 1923; Mason 1926). At death, these rats presented normal liver. The reason for this result is unknown. It may be that the wasting away of muscle releases sufficient of the sulfur containing amino acids to protect the liver.

Influence of lard and cod liver oil. Lard and cod liver oil are important variables in the induction of vitamin E sterility in rats.

The incidence of liver necrotic degeneration was not influenced by variations from 0 to 34 per cent in the lard content of the isocaloric diets. The results were not altered with three different types of lard: modern processed, old type or Eastman low E. In other diets, afaxin and viosterol were substituted for the cod liver oil. All of the rats developed fatal hepatic necrosis. It appears that the disease occurs in the absence of cod liver oil and is independent of the lard concentration of the diets, under the given conditions.

Variations in the content of casein. The protective effects of high casein diets are well known. The crude casein concentration was varied from 5.8 to 14.0 per cent in diets containing 6 per cent lard. All of the rats with the 5.8 per cent diet, 2 of 6 with 11.6 per cent casein, and none of 24 with 14.0 per cent casein acquired liver necrotic degeneration. Chronic muscular dystrophy appeared in all rats that did not develop liver lesions. They survived for 4 to 6 months, presenting severe muscular dystrophy and normal liver at death.

Other sources of protein. Under the given conditions, liver necrotic degeneration has been observed with low E—low protein diets (Goettsch, 1948, 1951).

— — — — — type yeast ally grown, ins contain

the factor 3 of Schwarz (1951) is not known.

Initial size of the low-E rat. Since it has been customary to use rats with an initial body weight of 70–100 gm, young low E rats were maintained with the

DIETARY HEPATIC NECROSIS IN THE RAT—ABSENCE OF CIRRHOSIS FOLLOWING RECURRENT EPISODES*

By F. W. Hoffbauer and Bernadine Wittenburg

Department of Medicine, The Medical School, University of Minnesota, Minneapolis, Minn

The investigations to be described arose from an interest in the human disease postnecrotic cirrhosis, and represent an attempt to produce an experimental form of the disease. This type of cirrhosis presents a challenge to the pathologist and to the clinician. The etiologic factor or factors responsible are obscure. The morbid anatomy is variable. The clinical course is, however, fairly constant. Patients with postnecrotic cirrhosis quite uniformly exhibit a gradual but progressive deterioration of hepatic function and the disease ultimately proves fatal. The clinical course and the rate of progression of the disease is subject to great individual variation. We are not convinced that

those do follow an attack of viral hepatitis

There is a similarity between one form of cirrhosis seen in the clinic (the patient who is a victim of chronic alcoholism and consequent malnutrition) and one form of experimental cirrhosis (the rat that is a victim of prolonged fatty infiltration of the liver). The investigator possesses a laboratory method by which he can produce a disorder that resembles in many respects a disease that he encounters in the hospital wards. In attempting the production of an experimental form of postnecrotic cirrhosis, an obvious approach would be to cause chronic viral hepatitis in an animal. This most desirable accomplishment seems quite unobtainable at the present time. Hence, we sought to produce a structural counterpart of this disorder by inducing liver damage through a mechanism that was available. The lack of any etiologic similarity between the method used, experimental dietary necrosis, and viral hepatitis was recognized and appreciated.

In the experimental production of any type of cirrhosis, repeated exposure to the insulting or damaging agent is an essential feature. When carbon tetrachloride is employed for this purpose, the investigator administers the toxic agent twice a week, three times a week, week after week, until he obtains the desired goal. In the case of fatty infiltration, the damaging effects of the fatty cysts proceed continuously night and day in the fashion that Hartroft¹ has demonstrated. Would it be possible to produce a structural counterpart of human postnecrotic cirrhosis if an animal could be forced to undergo repeated episodes of dietary liver necrosis? The following studies were directed toward such a goal. There are two aspects to be reported: (a) the resultant pathologic changes observed after the spontaneous occurrence of repeated attacks of dietary necrosis, (b) the resultant changes noted when one attempts to modify the course of events after one or more episodes of necrosis have occurred.

*These investigations were sponsored by the Commission on Liver Disease, Armed Forces Epidemiological Board and supported in part by a contract from the Office of the Surgeon General, U.S. Army.

(2) These lesions develop in the absence of cod liver oil and are independent of the lard content of isocaloric diets

(3) They occur in the same type and torul

(4) An increase in methionine, decreases the incidence of liver degeneration and permits the

tion enabling the rat to survive for a long period of time and to reproduce under conditions that are associated with fatty deposition in the liver and cirrhosis. Methionine prevents, and cystine intensifies, fatty infiltration of the liver and diffuse hepatic fibrosis

References

- BEST C H & M E HUNSMAN 1932 The effects of the components of lecithin upon deposition of fats in the liver J Physiol 75 403-412
- DART I S W H SEBELL & R D LILLIE 1942 Prevention by cystine or methionine of hemorrhage and necrosis in the liver of rats Proc Soc Exptl Biol Med 50 1-5
- EARLE D P & J VICTOR 1941 Cirrhosis of the liver caused by excess dietary cystine J Exptl Med 73 161-172
- ELVARSON A & A RINGSTED 1938 Effect of Chronic Vitamin E Deficiency on the Nervous System and Skeletal Musculature in Adult Rats Levin and Munksgaard Copenhagen
- EVANS H M & K S BISHOP 1923 The production of sterility with nutrition regimes adequate for growth and its cure with other foodstuffs J Metabolic Research 3 233-238
- GOETTSCH M 1948 Minimal protein requirement for growth in the rat Arch Biochem 19 349-358
- GOETTSCH M 1951 The role of vitamin E in the production of nutritional liver injury in rats on low casein diets J Nutrition 44 443-454
- GYÖRGY P 1947 Conference on Liver Injury Josiah Macy Jr Foundation NY 67
- GYÖRGY P & H GOLDBLATT 1949 Further observations on the production and prevention of dietary hepatic injury in rats J Exptl Med 89 245-268
- HAWK P B & B L OSER 1931 A modification of the Osborn Mendel salt mixture Science 74 369
- HIMSWORTH H P & O LINDAN 1949 Dietetic necrosis of the liver the influence of α tocopherol Nature 163 30
- HOVE E L D H COPELAND & W D SALL 1949 Dietetic necrosis of the liver characterized by massive lung hemorrhage J Biol Chem 188 1-10
- LINDAN O & H P HIMSWORTH 1950 The development of dietetic massive necrosis of the liver J Biol Chem 188 651-663
- MASON K E 1926 Testicular degeneration in albino rats fed a purified food ration J Exptl Zool 31 159-223
- SCHWARZ K 1944 Tocopherol as a liver protecting substance Z physiol Chem 281 106-116
- SCHWARZ K 1949 Dietetic hepatic injuries and the mode of action of tocopherol Ann N Y Acad Sci 52 225-230
- SCHWARZ K 1951 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 1952 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 1953 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 1954 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 1955 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 1956 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 1957 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 1958 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 1959 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 1960 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 1961 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 1962 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 1963 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 1964 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 1965 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 1966 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 1967 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 1968 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 1969 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 1970 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 1971 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 1972 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 1973 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 1974 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 1975 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 1976 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 1977 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 1978 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 1979 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 1980 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 1981 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 1982 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 1983 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 1984 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 1985 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 1986 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 1987 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 1988 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 1989 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 1990 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 1991 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 1992 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 1993 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 1994 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 1995 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 1996 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 1997 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 1998 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 1999 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 2000 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 2001 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 2002 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 2003 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 2004 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 2005 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 2006 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 2007 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 2008 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 2009 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 2010 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 2011 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 2012 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 2013 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 2014 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 2015 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 2016 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 2017 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 2018 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 2019 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 2020 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 2021 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 2022 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 2023 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 2024 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10
- SCHWARZ K 2025 The effect of tocopherol on the development of liver degeneration in rats J Biol Chem 191 1-10

TABLE 2

INCIDENCE OF POSTNECROTIC SCARRING OF THE LIVER IN RATS DYING OF DIETARY NECROSIS

No. of rats	Sex	Initial weight ($\pm 10^{\text{th}}$)	Diet*	Survival period (days)	Degree of Scarring		
					Absent	Minimal	Marked
10	M	50 gm	H2	34-72	5	4	1
15	F	50 gm	H2	27-74	11	4	3
10	M	100 gm	BY18	63-116	3	7	—
10	F	100 gm	BY18	64-144	5	4	—
9	M	100 gm	TY20	43-137	8	1	—
13	F	100 gm	TY20	74-202	8	5	—
3	M	100 gm	TY35	72-106	—	3	—
5	F	100 gm	TY35	118-180	3	2	—
17	M	50 gm	BY18 R	25-59	2	10	5
0	M	50 gm	BY18	28-70	21	32	17

* Diets: H2—18%, Baker's yeast, corn starch, and oil (see Text); BY18—18%, Baker's yeast, sucrose, and lard (see Text); TY20—20%, Torula yeast, sucrose, and lard (see Text); TY35—35%, Torula yeast, sucrose, and lard (see Text); BY18 R—1 ml. resin (Reson) added to daily ration (see Text).

completion of an experiment, all specimens were graded as to the degree of scarring. Appropriate sections were then removed for histologic study. A massive hemorrhagic necrosis of the liver was the cause of death in all rats except as described subsequently (TABLES 4 and 5). This lesion can be detected by gross inspection. The presence of scarring indicative of one or more previous episodes of submassive necrosis can also be detected by careful gross inspection. The scarring was graded as *minimal* if the left lateral lobe alone was involved and as *marked* if one or more additional lobes revealed the characteristic pits or furrows. Microscopic sections were prepared by standard procedures. Hematoxylin-eosin and azocarmine stains were used most frequently. In a few instances, plastic corrosion preparations of the intrahepatic veins were made by injecting colored vinyl acetate solutions into the portal and hepatic veins.

RESULTS

The presence of depressed scars in the liver of a rat dying of dietary liver necrosis is an indication that the animal has undergone one or more previous episodes of liver cell degeneration. The sequence of events that leads to the development of such lesions has been well described by Himmsworth⁴ and by Allen, Beveridge, and Fisher.⁵

The addition of the resin compound had no influence on the course of the disease or the resultant pathology (the 17 controls for experiment are included among 70 males in TABLE 2).

course.

TABLE 1
COMPOSITION OF DIET BY18

Sucrose	72%
Yeast ¹	18%
Salt ²	4%
	1%
	0.05%
	4.92%

¹ Dried Bakers Yeast, I. M. J. Co. N. Y.

² A special lard tocopherol content minimal †

* Kindly made available by Ayerst, McKenna and Harrison Ltd. New York 16 New York
† Kindly made available by Hormel Company, Austin 2 Minnesota

Experimental Methods Employed

Throughout these experiments, a uniform strain of white laboratory rats^{*} was used. Male rats weighing 50 ± 5 gm at the start of the experiment were routinely employed except in the initial investigations (described in TABLE 2). The animals were housed in individual cages suspended in racks of a type that provided a continuous sheet of semi waterproof paper beneath the cages. This arrangement proved convenient, since daily urine tests for bilirubin were necessary. Specimens for testing were secured from small pools of urine that collected beneath each cage. Franklin's² method for detecting bilirubin in the urine was employed routinely in these studies. Daily urine tests were begun 20 days after the animals were started on the necrogenic diet.

Diet. The diet employed in the major portion of this study was designated by BY18. The composition is given in TABLE 1. Each rat was allowed 8 gm. per day. Fresh rations were mixed once each week and refrigerated. The animals were fed three times each week. Food consumption was not routinely measured. Free access to water was allowed.

In preliminary investigations diets of slightly different composition were given as indicated in the previous report.²

35 percent Torula

only two respects. There was a reduction in the sucrose content to 10 and 50 per cent respectively. The vitamin mixture employed did not contain vitamin K. The diet BY18R differed only in that 1 ml. of a liquid resin preparation[†] was added to the 8 gm. ration allowance offered daily to each animal.

Pathological analysis. Animals were autopsied as soon after death as possible. Where the animals were obviously moribund they were anesthetized and sacrificed. The livers were fixed in a neutral formalin solution. At the

* Purchased from Holtzman Rat Company, Madison 5, Wisc.

† Supplied by the Sulphite Pulp Manufacturers Research League, Appleton, Wisc.

‡ RESIONR, supplied by the National Drug Co., Philadelphia, Pa.

TABLE 1
EXPERIMENT 1
INCIDENCE OF DEATHS IN RATS GIVEN EXPERIMENTAL DIET*

Group	Management		No. tested	No. dead with acute liver necrosis	Mean survival time (days)	No. dead without acute liver necrosis
	Housing: Usual range of temperature °F	Feeding				
A	Animal house (63-70°)	"Ad lib."	8	7	19	0
B	(heated)	Restricted	9	1†	—	0
C	Rabbit hutch (40-50°)	"Ad lib."	8	0	—	0
D	(unheated)	Restricted	9	0	—	4

* Survivors killed 33 days after feeding low-protein diet

† Rat died 33 days after feeding low-protein diet

died of acute liver necrosis while those with a restricted food allowance or those in the lower environmental temperature (40-50°F) did not develop the disease. When the food allowance was restricted in the lower environmental temperature four among nine rats died without liver necrosis.

The second and third experiments were conducted in the winter of 1949-1950 and in the spring of 1950 with better temperature control of the heated environments. In experiment 2 the usual range of the air temperature of the unheated environment was 40-50°F, that of the heated environment 70-74°F, i.e., several degrees Fahrenheit above the temperature studied in experiment 1. In experiment 3, groups of rats in two heated environments 70-74°F and 60-65°F, and two unheated environments were studied. In this experiment, only one 'restricted' group of rats was studied (group 3E TABLE 2). These rats were put into the environment of 60-65°F, so that the conditions at the beginning of experiment 1 were

reproduced. Those of experiment 1 who died of acute liver necrosis were confirmed those of experiment 3 who died of acute liver necrosis was the same among the rats "restricted" in their food allowance.

TABLE 2
EXPERIMENTS 2 AND 3
INCIDENCE OF DEATHS IN RATS GIVEN EXPERIMENTAL DIET

Experiment	Group	Management		No. of rats tested	No. dead with acute liver necrosis	Mean survival time (days)	No. dead without liver necrosis
		Housing: Usual range of temperature °F	Feeding				
1	A	Animal house (70-74)	"Ad lib."	9	8	43	0
	B	(heated)	Restricted	9	3	40	0
	C	Unheated wooden shed (40-50)	"Ad lib."	10	3	—	0
	D	(unheated)	Restricted	10	1	—	3
3	A	Animal house (70-74)	"Ad lib."	12	11	19	0
	B	Heated wooden shed (60-65)	"Ad lib."	12	8	26	0
	C	(heated)	Restricted	12	0	—	1†
	D	Rabbit hutch (35-45)	"Ad lib."	12	0	—	0
	E	Heated wooden shed (60-65)	Restricted	8	0	—	0

† Died because of severe otitis media on 21st day after beginning of experimental diet

THE EFFECT OF ENVIRONMENT AND MODE OF FEEDING AND OF REARING ON THE PRODUCTION OF ACUTE DIETARY LIVER NECROSIS IN THE RAT

By J M Naftalin

Rowett Research Institute, Bucksburn, Aberdeenshire Scotland

My aim in the experiments described below was to define the conditions under which young male rats would become susceptible to acute dietary liver necrosis

In the first series of five experiments,¹⁻⁵ two conditions were examined simultaneously, (1) the influence of environment, and (2) the mode of feeding; i.e. whether the rats were allowed to eat to appetite or not. In the second series of three experiments³ a method was found of adjusting the susceptibility of groups of rats to the development of acute liver necrosis. With such a method more detailed comparisons can be made between rats likely to develop liver necrosis in a short time and those which would take longer to do so or would not develop the disease at all.

The Interactions between Environmental Temperature and Mode of Feeding

temperature has been examined in detail. Briefly the usual ranges of environmental temperature were 35-45°F, 40-50°F, 60-64°F, 63-70°F, 70-74°F, 72-76°F, 70-78°F, and 88-92°F.

Diets The following three diets were used. (1) During pregnancy and the first 17 or 19 days of lactation, the Rowett Research Institute stock rat cubes⁶ together with cow's milk was given. This diet was followed by (2) a semi-synthetic preliminary high protein diet containing casein 16 or 19 (vitamin free)⁷ Glaxo Laboratories Ltd, Middlesex, England), brewers' yeast 3 (Phar

Chemical Products, Ltd, London), sucrose 68 or 65 commercial lard 7
4, cod liver oil (B.P.) 2 per cent, and synthetic B vitamins but
Vitamin E. These diets were available to the young (3)
week of age, the young were fed a low protein diet containing
of protein, 8 per cent casein and 3 per cent yeast, the sucrose
ed to 76 per cent. The yeast was added to supply the extra
red by animals exposed to cold. The reasons for
lained in a previous paper.¹

Experiments The rats were weaned on the 24th day of
cub diet was offered, one group of rats in each environ
to eat to appetite and, except in experiment 3, one or two
environment were restricted in different degrees in their food in

Results

are shown in TABLE 1. It is evident that
perature of 63-70°F the rats which ate to

THE EFFECT OF ENVIRONMENT AND MODE OF FEEDING AND OF REARING ON THE PRODUCTION OF ACUTE DIETARY LIVER NECROSIS IN THE RAT

By J M Naftalin

Rowett Research Institute, Bucksburn, Aberdeenshire, Scotland

My aim in the experiments described below was to define the conditions under which young male rats would become susceptible to acute dietary liver necrosis

In the first series of five experiments,¹⁻⁵ two conditions were examined simultaneously, (1) the influence of environment, and (2) the mode of feeding, i.e., whether the rats were allowed to eat to appetite or not. In the second series the susceptibility of the rats was examined at different temperatures. With such a method, it was possible to develop liver

necrosis in a short time and those which would take longer to do so, or would not develop the disease at all

The Interactions between Environmental Temperature and Mode of Feeding

Environments The environments are described in detail in previous papers.¹⁻⁵ It should be emphasized that, although these experiments were designed in the first instance to study the environment, only the effect of the dry bulb air temperature has been examined in detail. Briefly the usual ranges of environmental temperature were 35-45°F, 40-50°F, 60-64°F, 63-70°F, 70-74°F, 72-76°F, 70-78°F, and 88-92°F

Diets The following three diets were used. (1) During pregnancy and the first 17 or 19 days of lactation, the Rowett Research Institute stock rat cube⁶ together with cow's milk was given. This diet was followed by (2) a semi-synthetic preliminary high protein diet containing casein 16 or 19 ("vitamin free" Glaxo Laboratories Ltd, Middlesex, England), brewers' yeast 3 (Pharmaco-Chemical Products, Ltd, London), sucrose 68 or 65, commercial lard 7, McCollum's salts 4, cod liver oil (B.P.) 2 per cent, and synthetic B vitamins but without added Vitamin E. These diets were available to the young. (3) From the sixth week of age, the young were fed a low protein diet containing, as a source of protein, 8 per cent casein and 3 per cent yeast, the sucrose being increased to 76 per cent. The yeast was added to supply the extra energy which may be required by animals exposed to cold. The reasons for this are explained in a previous paper.¹

Experiments The rats were weaned on the 24th day of age. The low protein diet was offered, one group of rats in each environment being allowed to eat to appetite and except in experiment 3, one or two groups were restricted in different degrees in their food intake.

Results

The results of the experiments are shown in TABLE 1. It is evident that at a temperature of 63-70°F the rats which ate to appetite

TABLE 1
EXPERIMENT 1
INCIDENCE OF DEATHS IN RATS GIVEN EXPERIMENTAL DIET*

Group	Management		No. tested	No. dead with acute liver necrosis	Median survival time (days)	No. dead without acute liver necrosis
	Housing: Usual range of temperature °F	Feeding				
A	Animal house (63-70°)	'Ad lib'	8	7	19	0
B	(heated)	Restricted	9	1†	—	0
D	Rabbit hutch (40-50°)	'Ad lib'	8	0	—	0
E	(unheated)	Restricted	9	0	—	4

* Survivors killed 23 days after food of low protein diet

† Rat died 12 days after feeding low-protein diet

died of acute liver necrosis while those with a restricted food allowance or those in the lower environmental temperature (40-50°F) did not develop the disease. When the food allowance was restricted in the lower environmental temperature, four among nine rats died without liver necrosis.

The second and third experiments were conducted in the winter of 1949-1950 and in the spring of 1950 with better temperature control of the heated environments. In experiment 2 the usual range of the air temperature of the unheated environment was 40-50°F, that of the heated environment 70-74°F, i.e., several degrees Fahrenheit above the temperature studied in experiment 1. In experiment 3 groups of rats in two heated environments 70-74°F and 60-65°F

formed those of experiment 3, while those of experiment 2 differed in two main respects: (1) in the heated environment of 70-74°F, the incidence of acute liver necrosis was the same among the rats 'restricted' in their food allowance

TABLE 2
EXPERIMENTS 2 AND 3
INCIDENCE OF DEATHS IN RATS GIVEN EXPERIMENTAL DIET

Experiment	Group	Management		No. of rats tested	No. dead with acute liver necrosis	Median survival time (days)	No. dead without liver necrosis
		Housing: Usual range of temperature °F	Feeding				
1	A	Animal house (70-74)	'Ad lib'	9	8	43	0
	B	(heated) wooden shed	Restricted	9	8	40	0
	C	(unheated) wooden shed (40-50)	'Ad lib'	10	3	—	0
	D	(40-50)	Restricted	10	1	—	1
	A	Animal house (70-74)	'Ad lib'	12	11	19	0
	B	Heated wooden shed (65-68)	'Ad lib'	12	8	26	0
2	A	Rabbit hutch (65-68)	'Ad lib'	12	0	—	1†
	B	Heated wooden shed (65-68)	Restricted	8	0	—	0

† Rat became ill severely on 21st day of experiment but recovered on the experimental diet.

OF ENVIRONMENT AND MODE OF FEEDING AND ON THE PRODUCTION OF ACUTE DIETARY LIVER NECROSIS IN THE RAT

By J M Naftalm

Research Institute, Bucksburn Aberdeenshire, Scotland

described below was to define the conditions under which rats would become susceptible to acute dietary liver necrosis. In five experiments,¹⁻⁵ two conditions were examined: (1) the influence of environment, and (2) the mode of feeding; i.e., whether rats were allowed to eat to appetite or not. In the second series of experiments,³ a method was found of adjusting the susceptibility of rats to the development of acute liver necrosis. With such a method, direct comparisons can be made between rats likely to develop liver necrosis in a short time and those which would take longer to do so, or would not develop the disease at all.

The Interactions between Environmental Temperature and Mode of Feeding

Environments The environments are described in detail in previous papers.¹⁻⁵ It should be emphasized that, although these experiments were designed in the first instance to study the environment, only the effect of the dry bulb air temperature has been examined in detail. Briefly the usual ranges of environmental temperature were 35-45°F, 40-50°F, 60-64°F, 63-70°F, 70-74°F, 72-76°F, 70-78°F, and 88-92°F.

Diets The following three diets were used: (1) During pregnancy and the first 17 or 19 days of lactation, the Rowett Research Institute stock rat cube¹ together with cow's milk was given. This diet was followed by (2) a semi-synthetic preliminary high protein diet containing casein 16 or 19 ("vitamin free" Glaxo Laboratories Ltd, Middlesex, England), brewers' yeast 3 (Pharmaco Chemical Products, Ltd, London), sucrose 68 or 65, commercial lard 7, McCollum's salts 4, cod liver oil (B.P.) 2 per cent, and synthetic B vitamins but without added Vitamin E. These diets were available to the young. (3) From the sixth week of age, the young were fed a low protein diet containing as the source of protein, 8 per cent casein and 3 per cent yeast, the sucrose being increased to 76 per cent. The yeast was added to supply the extra vitamins which may be required by animals exposed to cold. The reasons for these procedures are explained in a previous paper.¹

Management of the experiments The rats were weaned on the 24th day of age. When the low protein diet was offered, one group of rats in each environment was allowed to eat to appetite and except in experiment 3, one or two groups in each environment were restricted in different degrees in their food intake.

Results

The results of the first experiment are shown in TABLE 1. It is evident that in an environmental temperature of 63-70°F the rats which ate to appetite

TABLE 1
EXPERIMENT 1
INCIDENCE OF DEATHS IN RATS GIVEN EXPERIMENTAL DIET*

Group	Management		No. tested	No. dead with acute liver necrosis	Median survival time (days)	No. dead without acute liver necrosis
	Housing (usual range of temperature $^{\circ}\text{F}$)	Feeding				
A	Animal house (63-70 $^{\circ}$) (heated)	Ad lib.	8	7	19	0
B		Restricted	9	1†	—	0
D	Rabbit hutch (40-50 $^{\circ}$) (unheated)	Ad lib.	8	0	—	0
E		Restricted	9	0	—	4

* Survivors killed 33 days after feeding low protein diet.

† Rat died 33 days after feeding low protein diet.

died of acute liver necrosis while those with a restricted food allowance or those in the lower environmental temperature (40-50 $^{\circ}\text{F}$) did not develop the disease. When the food allowance was restricted in the lower environmental temperature, four among nine rats died without liver necrosis.

The second and third experiments were conducted in the winter of 1949-1950 and in the spring of 1950 with better temperature control of the heated environments. In experiment 2 the usual range of the air temperature of the unheated environment was 40-50 $^{\circ}\text{F}$, that of the heated environment 70-74 $^{\circ}\text{F}$, i.e., several degrees Fahrenheit above the temperature studied in experiment 1.

of experiment 1 were simulated. The results (TABLE 2) of experiment 3 confirmed those of experiment 1, while those of experiment 2 differed in two main respects: (1) in the heated environment of 70-74 $^{\circ}\text{F}$, the incidence of acute liver necrosis was the same among the rats restricted in their food allowance

TABLE 2
EXPERIMENTS 2 AND 3
INCIDENCE OF DEATHS IN RATS GIVEN EXPERIMENTAL DIET

Experiment	Group	Management		No. of rats tested	No. dead with acute liver necrosis	Median survival time (days)	No. dead without liver necrosis
		Housing (usual range of temperature $^{\circ}\text{F}$)	Feeding				
2	A	Animal house (70-74)	Ad lib.	9	8	43	0
	B		Restricted	9	8	40	0
	C	Unheated wooden shed (40-50)	Ad lib.	10	3	—	0
	D		Restricted	10	1	—	3
3	A	Animal house (70-74)	Ad lib.	12	11	19	0
	B		Ad lib.	12	8	26	0
	D	Heated wooden shed (60-65)	Ad lib.	12	0	—	1†
	E		Restricted	11	0	—	0

† Killed because of severe diarrhea on 2nd day after beginning on the experimental diet.

THE EFFECT OF ENVIRONMENT AND MODE OF FEEDING AND OF REARING ON THE PRODUCTION OF ACUTE DIETARY LIVER NECROSIS IN THE RAT

By J M Naftalin

Rowett Research Institute Bucksburn Aberdeen Scotland

My aim in the experiments described below was to define the conditions under which young male rats would become susceptible to acute dietary liver necrosis

In the first series of five experiments,¹⁻⁵ two conditions were examined simultaneously, (1) the influence of environment, and (2) the mode of feeding i.e. whether the rats were allowed to eat to appetite or not. In the second series of three experiments,³ a method was found of adjusting the susceptibility of groups of rats to the development of acute liver necrosis. With such a method more detailed comparisons can be made between rats likely to develop liver necrosis in a short time and those which would take longer to do so or would not develop the disease at all.

The Interactions between Environmental Temperature and Mode of Feeding

Environments The environments are described in detail in previous papers.¹⁻⁵ It should be emphasized that, although these experiments were designed in the first instance to study the environment, only the effect of the dry bulb air temperature has been examined in detail. Briefly the usual ranges of environmental temperature were 35-45°C, 40-50°C, 60-64°F, 63-70°F, 70-74°F, 72-76°F, 70-78°F, and 88-92°F.

Diets The following three diets were used. (1) During pregnancy and the first 17 or 19 days of lactation, the Rowett Research Institute stock rat cube⁶ together with cow's milk was given. This diet was followed by (2) a semi-

16-20 (vitamin
st 3 (Pbar
cial lard 7
itamins but
oung (3)

From the sixth week of age, the young were fed a low protein diet containing as the source of protein, 8 per cent casein and 3 per cent yeast, the sucrose being increased to 76 per cent. The yeast was added to supply the extra vitamins which may be required by animals exposed to cold. The reasons for these procedures are explained in a previous paper.¹

Management of the experiments The rats were weaned on the 24th day of age. When the low protein diet was offered, one group of rats in each environment was allowed to eat to appetite and except in experiment 3 one or two groups in each environment were restricted in different degrees in their food intake.

Results

The results of the first experiment are shown in TABLE 1. It is evident that in an environmental temperature of 63-70°F the rats which ate to appetite

TABLE 4
RESULTS OF FOOD RESTRICTION AT VARIOUS ENVIRONMENTAL TEMPERATURES
(Experiments 1-5)

Environmental temperature $^{\circ}\text{F}$ (usual range)	Expt. no.	Group average degree of food restriction	Results (restricted groups)
88-92	5	1.7 1.2	Death without liver necrosis Protection against liver necrosis Rats live
70-78	4	1.6	Not complete protection against liver necrosis
60-74	2	1.3	No protection against liver necrosis
60-64	4	1.2-1.1	No protection against liver necrosis
	4	1.6	Death without liver necrosis
	3	1.6-1.4 (lowest 1.25)	Protection against liver necrosis Rats live
	4	1.2	No protection against liver necrosis
About 63	1	1.15, 1.09*	Protection against liver necrosis.
Below about 45	1 & 2	When ratio was about 1.35	Death without liver necrosis.

* Only 2 rats studied for one week in the corresponding "4d Lab" group

at the higher environmental temperature of 70-78 $^{\circ}\text{F}$, the rats which were

degree of food restriction was 1.2 in the environmental temperature of 60-64 $^{\circ}\text{F}$. The degree of food restriction necessary to prevent death at 60-64 $^{\circ}\text{F}$ would seem to lie about 1.4—the ratio used in experiment 3 (TABLE 4).

Experiment 5 was an extension of these observations. Littermates were

ment. From this experiment it was concluded that an environmental temperature of 88-92 $^{\circ}\text{F}$ was higher than the optimum temperature for the production of acute liver necrosis.² This conclusion was reached because, at this temperature the group of rats allowed to eat to appetite survived longer than their littermates housed at 74-76 $^{\circ}\text{F}$, and because the low degree of food restriction of 1.2 prevented liver necrosis. The range 80-85 $^{\circ}\text{F}$ has not yet been studied but an environmental temperature of about 75 $^{\circ}\text{F}$ is suitable for a high incidence of the disease. It was thus apparent that it was the degree of food restriction in relation to the environmental temperature that determined whether a rat survived or died either of liver necrosis or of inanition (TABLE 4). Although the trend is clear it should not be inferred that these ratios are fixed

TABLE 3

EXPERIMENTS 1, 2, AND 3

FOOD EATEN BY "AD LIB" GROUPS* AND DEGREE OF FOOD RESTRICTION

Expt no	Environ temp °F Usual range (extreme range)	Average food eaten g./rat/day by "ad lib" group ()	Average food offered g./rat/day to restricted group ()	Average degree of food restriction ad lib/restricted	Remarks
1	63-70	5.8 (A)	5.0 (B)	1.15, 1.09	1. Only 2 rats studied for 1 week in "ad lib" group A. 2. One rat out of 6 died with liver necrosis in restricted group II when environmental temp reached 80°F.
	40-50	11.8 (D)	8.8 (E)	1.3	Four rats died without liver necrosis in restricted group E. Degree of restriction greater than 1.3 at that time.
2	70-74	4.5→3.9 (A)	3.6 (B)	1.2→1.1	Incidence of liver necrosis the same in groups A and B, 8 out of 9.
	40-50	10.8 (E)	7→9.4 (F)	1.2	Three rats died without liver necrosis in group F when degree of restriction was 1.4, 1.34 and 1.35. Temps 38, 28, and 40°F.
3	60-65	7.0 (B)	4→6 (E)	1.6→1.4	No rat died in restricted group E.

* Only groups relevant to the argument are shown. For details of other groups see Naftalin.
 → = increased or reduced as experiment progressed

(group 2B TABLE 2) as among those fed "ad lib" (group 2A), (2) the median survival times, after first giving the experimental low protein diet, were 40 and 43 days, as compared with 17 and 19 days in experiments 1 and 3. The reason for this second difference is not fully apparent, though it may be due to seasonal changes.

In seeking to explain why the "restricted" rats in experiment 2 behaved differently from the "restricted" rats in experiments 1 and 3, the amounts of food eaten and the degree of food restriction were then examined. The results are shown in TABLE 3. In experiment 2, the rats fed "ad lib" (group 2A) did not eat as much as those in corresponding groups in experiments 1 or 3 (groups 1A and 3B). The average amount eaten by group 2A decreased from 4.5 to 3.9 gm./rat/day as the experiment progressed. It had been decided at the beginning of experiment 2 to offer 3.8 gm. of food daily to each rat in group 2B, the "restricted" group but, although after 11 days this was reduced to 3.6 gm., the ratio of food eaten by the group fed "ad lib" to the "restricted" group did not rise above 1.1. It seemed, therefore, that the degree of food restriction had not been great enough at 70-74°F to prevent death from acute liver necrosis. This postulate was tested in experiment 4, when two degrees of food restriction were studied at two environmental temperatures, 60-64°F and 70-78°F. The degrees of food restriction were such that the amounts of food

EFFECT OF DIFFERENT MAKES OF CASEIN ON THE PRODUCTION OF ACUTE DIETARY LIVER NECROSIS IN THE RAT

By J M Naftalin

Robert Research Institute, Bucksburn, Aberdeenshire, Scotland

From 1949 till the summer of 1951, the casein used in diets to induce acute liver necrosis in rats was the "vitamin free" product of Glaxo Laboratories, Ltd, Middlesex, England. When this casein became unavailable commercially, "low vitamin" content casein of Genatosan, Ltd, Loughborough, England, was substituted. The next two experiments were conducted in the autumn of 1951 under those conditions which had been found to be most favorable for the production of the disease, *i.e.*, when the rats were housed in an environmental temperature of about 75°F and allowed to eat to appetite. In these latter experiments,¹ no rat suffered from liver necrosis. This paper reports in more detail the results obtained by a direct comparison, using litter-mates, of two batches of casein, Genatosan casein "low vitamin" content, batch no. 078689, supplied in 1951, and Glaxo lactic casein "C" (unextracted), batch no. U4529, supplied in 1947. It was not possible to make a direct comparison with the sample of Glaxo "vitamin free" casein used in previous experiments.

Methods

Two experiments lasting from February to April, 1952 and from April to July, 1952 are described. The environmental temperature of the animal house was 74°-78°F. Young male rats of the hooded Lister strain, Rowett Institute stock, were weaned on their 17th day, instead of their 24th day, as is customary in this Institute, as it had been found that groups with a higher degree of sensitivity to liver necrosis were thus obtained.^{1, 2}

The diets contained casein 16 (Glaxo lactic casein "C" unextracted or Genatosan casein "low vitamin" content) brewers yeast 3 (Pharmaco-Chemical Products Ltd, London) sucrose 68 commercial lard 7 McCollum's salts 4 cod liver oil (B.P.) 2 per cent choline chloride 200 mgm/100 gm diet, and B vitamins but without added vitamin F. These diets were offered from the 17th to 34th day of age and were followed on the 35th day of age by diets in which the casein was reduced to 8 per cent and the sucrose increased to 76 per cent. The rats were always allowed to eat to appetite.

a week from their 34th day of age. Group C was given diets containing Glaxo lactic casein "C". In experiment 2 six male rats from each of twelve litters were allocated at random to 2 groups D and E. Three rats from each litter were put into each group and fed on diets containing either Genatosan casein or Glaxo lactic casein "C".

The reasons for the differences between the rats weaned early or late are under investigation. Since the preliminary high protein diet fed to the mothers from the 17th day of lactation may in itself lead to necrosis in the young, it would seem that the rats weaned later either (1) had a nonspecific improvement in their nutrition or (2) they received more of a protective substance which, (a) although present only in traces in the diet, could be concentrated in the milk or which (b) was synthesized in the mother's body and secreted in the milk.

Summary

- (1) A high incidence of acute liver necrosis was produced in young male

cent lard 2 per cent cod liver oil (B P), 76 per cent sucrose, and 4 per cent McCollum's salt and B vitamins but without added vitamin E

(2) Of the ranges of environmental temperature studied the best temperature for the production of acute liver necrosis was 70°-78°F

(3) At all environmental temperatures studied the incidence of acute liver necrosis was reduced or prevented by a sufficient degree of food restriction but too severe a degree of food restriction led to death without liver necrosis. Whether a rat survived or died either of necrosis or of inanition depended on the degree of food restriction in relation to the environmental temperature and not simply on the total amount of food eaten

(4) Groups of rats weaned on their 17th day of age suffered a higher incidence of acute liver necrosis and had a shorter survival time than their littermates weaned on their 25th day

References

- 1 NAFTALIN J M 1951 J Path Bact 63 649
- 2 NAFTALIN J M 1952 Lancet 2 1013
- 3 NAFTALIN J M 1953 1954 J Path Bact In Press
- 4 SENGUPTA S B & J W HOWIE 1948 1949 Brit J Nutrition 2 313
- 5 ANDERSON R J & G LUSK 1917 J Biol Chem 30 421
- 6 BRODY S 1945 Bioenergetics and Growth New York 65
- 7 NAFTALIN J M 1953 Ann N Y Acad Sci 57 (6)

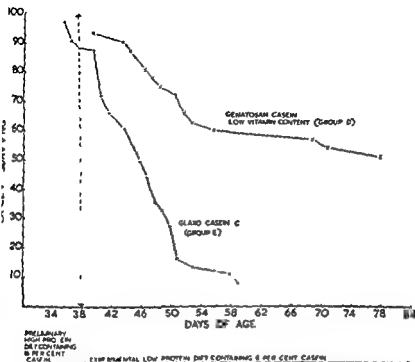


FIGURE 1. Graph of experiment 3. Percentage of rats surviving when fed on diets containing either Genatosan casein 'low vitamin' content or Glaxo casein 'C'. In group D (Genatosan casein) one rat died on 3rd day of age from lung hemorrhage; one rat died on 6th day of age from severe diarrhea and one rat was killed on 6th day of age. None of these rats had liver necrosis and are omitted from the calculation of percentage surviving. In group E, Glaxo casein 'C', four rats died from liver necrosis before the low protein diet was fed.

origin⁶ Genatosan casein 'low vitamin' content prepared by acid precipitation followed by extraction with methylated spirits and ether, is usually made from British liquid milk, although reconstituted dried skim milk of New Zealand origin is sometimes added if a sufficient quantity of liquid milk is not available.⁷ In two earlier experiments² when no liver necrosis resulted, an unnumbered batch of Genatosan casein 'low vitamin' content was used. In the experiments 1 and 2 of the present paper batch No 078689 of Genatosan casein 'low vitamin' content was made from a mixture of milks of British and New Zealand origin. Unfortunately no information is now available about the unnumbered batch of the earlier experiments. The differences between caseins prepared from British or New Zealand milk may be due to age or to differences arising in preparation or to differences in the milks themselves, which may arise from differences in the feeding systems.

That caseins differ in their ability to lead to liver necrosis was first reported by Schwarz in 1944.⁸ Despite this, many workers have omitted to mention the brand of casein they employ. Schwarz's casein VI which induced liver necrosis may have been regarded as specially treated casein because he prepared

TABLE 1
INCIDENCE OF ACUTE LIVER NECROSIS IN EXPERIMENTS 1 AND 2

Expt no.	Group	Make of casein used in diets	No of rats tested	No dead with acute liver necrosis†	Median survival time (days)‡	Remarks	
1	A	Genatosan* casein "low vitamin" content	2	=10	4	—	One rat died on each of the following days after feeding experimental diet, 11, 12, 24, 25
	B	Genatosan casein "low vitamin" content + vit B ₁₂ **	2	from each of 5 litters =10	1	—	One rat died 8 days after feeding experimental diet
	C	Glaxo‡ lactie casein "C" unextracted	2	=10	9	7	Two rats died on each of the following days after feeding experimental diet, 4, 5, 7 and three on the 13th day
2	D	Genatosan casein "low vitamin" content	3	from each of 12 litters =36	16	—	See graph
	E	Glaxo lactie casein "C" unextracted	3	=36	33	9	

* Genatosan Ltd. Loughborough, Leicestershire, England.

** Vitamin B₁₂: Cyanocobalamin (Glaxo) 0.5 µg in 0.1 ml. water given by mouth 3 times a week from 10th day

‡ Glaxo Laboratories Ltd. Greenford, Middlesex, England

† Days after feeding experimental diet: 5 = 5 days after 10th day of age. Survivors killed on 47th day in experiment 1 and on 72nd day in experiment 2

Results and Discussion

The

this vitamin was given to one group of ten rats (group B - TABLE 1). The addition of this vitamin may have delayed necrosis, but the numbers studied were too small for the result to be statistically significant.

In experiment 2, among the rats fed on the diet containing Glaxo lactie casein "C" (group E), 2 died from acute liver necrosis on their 36th day of age, another on the 37th day.

per cent of his casein VI obtained a high incidence of liver necrosis. In an earlier experiment,⁵ in which a diet containing 19 per cent Glaxo "vitamin free" casein was used, one rat among five died from acute liver necrosis. The results of experiment 2 are further evidence that a high level of protein per se in the diet does not necessarily prevent dietary liver necrosis.

Glaxo lactie casein "C" batch UA529 was a crude product of New Zealand

liver necrosis. As a result of their studies with a water washed casein and an oxidized casein, Hove, Copeland, and Salmon⁸ came to the conclusion that acute liver necrosis or massive lung hemorrhage in rats depended on a simultaneous deficiency of Vitamin E and a casein co factor. More recently Schwarz^{9, 10} has reported that a substance ("Factor 3") which prevents liver necrosis is present in a variety of natural materials including American crude casein and "vitamin free" casein prepared by repeated alcohol extraction. It should be emphasized that Glaxo lactic casein "C" (unextracted) is a crude product and therefore, it is not possible to state without further investigation whether the difference between its liver necrogenic properties and those of Genatosan casein 'low-vitamin' content is due to a difference in content of "Factor 3" or whether there is some other reason.

Summary

(1) There was a higher incidence of acute liver necrosis amongst rats housed in an environmental temperature of 74-78°F and allowed to eat to appetite when the casein in the diet was the lactic casein "C" (unextracted) of Glaxo Laboratories Ltd, Middlesex, England, than when the 'low vitamin' content casein of Genatosan Ltd, Loughborough England, was used.

(2) Glaxo lactic casein 'C' (unextracted) is a crude product of New Zealand origin. Genatosan casein "low vitamin" content is prepared from British liquid milk with or without the addition of reconstituted dried skim milk of New Zealand origin.

Acknowledgments

I am indebted to Dr W. F. J. Cuthbertson of Glaxo Laboratories Ltd, to Mr Todd of Genatosan Ltd, for information about the preparations used in the experiments described in this paper.

References

- 1 J. M. 1954 J. Path. Bact. In press
- 2 J. M. 1953 Ann. New York Acad. Sci. 67 (6)
- 3 UGGA P. & H. GOLDBLATT 1959 J. Exptl. Med. 70 185
- 4 WARR H. 1944 Z. physiol. Chem. 281 101
- 5 NAFTALIN J. M. 1951 J. Path. Bact. 63 649
- 6 CUTHBERTSON W. F. J. 1952 Personal communication
- 7 TODD F. A. 1952
- 8 HOVE, E. I. D. H. Nutrition
- 9 SCHWARZ, K. 1951
- 10 SCHWARZ, K. 1952

THE INFLUENCE OF THE ENDOCRINE GLANDS ON THE DEVELOPMENT OF ACUTE MASSIVE LIVER NECROSIS

By J. M. R. Beveridge
Queen's University Kingston, Ontario

The first indication that the endocrine glands probably had some influence on the development of dietary liver necrosis was given in 1935, by Weichselbaum¹ in his paper on the nutritional requirement of the rat for methionine and cystine. The lethal liver lesion that was produced in his rats on a diet low in these amino acids was almost undoubtedly acute massive hepatic necrosis, although no histological description of the tissue was given by him. In this publication, he refers to the fact that the mortality was definitely less in the females. Presumably, this difference is due directly or indirectly to the action of the sex glands. Similar observations have been made by Gyorgy² and by our group.³ Our results, utilizing rats of average initial weight 129 gm. and in an experiment terminated after 165 days are shown in TABLE 1. Although the mortality is essentially the same, the average time at which death occurred is significantly longer in the females.

An interesting observation has been made by Ferret⁴ to the effect that diets designed to induce liver necrosis caused continuous oestrus in ovariectomized animals in which 5 mgm. estradiol had been implanted into the spleen. The addition of cystine to the diet restored the ability to inactivate the estrogen. On the other hand, alpha-tocopherol did not have a similar action, and it must be concluded on the basis of this work at least that the inability of animals to inactivate estrogen has a negligible significance in the pathogenesis of liver necrosis.

During an investigation of hepatic damage caused by the feeding of alkali-treated casein to rats, Schwarz⁵ in 1944 noted that an extract of lipids from the adrenals inhibited the development of the lesion.

In 1951, the same worker⁶ reported the results of further studies on the role of the adrenals in the development of the type of dietary massive liver necrosis currently being discussed. Proceeding on the assumption that lack of glycogenesis is one of the essential features of liver degeneration and recalling the role of adrenal cortical secretion in promoting this process, he tested the effect of cortisone on rats being fed a basal necrogenic diet low in methionine-cystine and alpha-tocopherol.

The survival time was significantly and equally prolonged in both males and females, but there was no difference in the mortality due to massive liver damage (TABLE 2). This investigation showed clearly that cortisone ameliorated the necrogenic effect of the basal diet.

However, the assumption that imperfect liver glycogenesis is initially involved in dietary liver necrosis is not supported by some of our previous work. One of the first investigations carried out by us⁷ on entering into this field was a study of progressive structural and biochemical changes occurring in the liver prior to and at the time of the acute necrotic episode. No change in

TABLE 1
EFFECT OF INITIAL WEIGHT AND OF SEX ON THE DEVELOPMENT OF LIVER NECROSIS

	Sex	Rats that died of necrosis or were killed when moribund		Rats killed at 105 days		Total incidence of necrosis	
		Rats	Days on diet (av. range)	No. showing signs of necrosis	No. showing no signs of necrosis	No. of rats	Per cent
Y	M	16	58 (35-97)	3	1	19/20	95
YF	F	16	79 (48-103)	—	4	16/20	80

TABLE 2
RESPONSE OF MALES AND FEMALES TO CORTISONE (NO. OF ANIMALS IN PARENTHESES)

	Average survival time (in days)	
	Males	Females
Without cortisone	41.5 (6)	50.1 (4)
With cortisone	57.0 (5)	65.2 (4)
Difference due to cortisone	15.5 days	15.1 days

From Schwarz⁸

curred in liver glycogen levels until at the time of the acute episode when one would expect, the glycogen levels dropped essentially to zero. Apparently liver glycogenesis proceeds unimpaired until signs of hepatic damage appear.

Although the nature of the effect of the thyroid hormone on the development of liver damage due to a variety of causes in both experimental animals and in humans is controversial, there is complete agreement on the nature of the effect of the thyroid secretion on the development of acute massive liver necrosis seen in rats fed diets low in alpha tocopherol and in the sulfur containing amino acids. Handler and Follis⁸ first demonstrated the potentiating effect of desiccated thyroid when it was added to a basal necrogenic diet.

The diet used by these investigators contained 0.2 per cent desiccated thyroid and caused acute liver damage in about 50 per cent of 50 gm. male rats at an average interval of 40 days compared to an incidence of 100 per cent in 70 days for similar rats on the basal diet. The diet (TABLE 3) used by us¹⁰ contained

1.00 per cent necrosis after the basal diet
thyroid tissue on
lithiouracil on

the other hand, is not so clear cut. With a level of 0.5 per cent propylthiouracil the rats died at an average interval of 47 days but they showed no evidence of hepatic injury at death. It was assumed that the toxicity of the supplement was responsible for the death of the animals. Apparently, Handler and Follis had a similar experience with thiouracil but the time at which their animals died was not specified. At a level of only 0.05 per cent of propylthiouracil its toxic effect was sufficient to permit three of the animals to survive the

TABLE 1

EFFECT OF INITIAL WEIGHT AND OF SEX ON THE DEVELOPMENT OF LIVER NECROSIS

	Sex	Rats that died of necrosis or were killed when moribund		Rats killed at 105 days		Total incidence of necrosis	
		Rats	Days on diet (av range)	No showing signs of necrosis	No showing no signs of necrosis	No. of rats	Per cent
Y	M	16	88 (35-97)	3	1	19/20	95
Y F	F	16	79 (48-103)	—	4	16/20	80

TABLE 2

RESPONSE OF MALES AND FEMALES TO CORTISONE (% OF ANIMALS IN PARENTHESES)

	Average survival time (n days)	
	Males	Females
Without cortisone	41.5 (6)	50.1 (4)
With cortisone	57.0 (5)	65.2 (4)
Difference due to cortisone	15.5 days	15.1 days

From Schwarz⁶

current in liver glycogen levels until at the time of the acute episode when as one would expect, the glycogen levels dropped essentially to zero. Apparently liver glycogenesis proceeds unimpaired until signs of hepatic damage appear.

Although the nature of the effect of the thyroid hormone on the development of liver damage due to a variety of causes in both experimental animals and in humans is controversial, there is complete agreement on the nature of the effect of the thyroid secretion on the development of acute massive liver necrosis seen in rats fed diets low in alpha tocopherol and in the sulfur-containing amino acids. Handler and Folli⁸ first demonstrated the potentiating effect of desiccated thyroid when it was added to a basal necrogenic diet.

The diet used by these investigators contained 0.2 per cent desiccated thyroid and caused acute liver damage in about 50 per cent of 50 gm. male rats at an average interval of 40 days compared to an incidence of 100 per cent in 70 days for similar rats on the basal diet. The diet (TABLE 3) used by us¹⁰ contained 0.3 per cent desiccated thyroid and (TABLE 4) caused 88 per cent necrosis after an average interval of only 20 days or half the time required by the basal diet 39 days. These results are indicative of a profound effect of thyroid tissue on the development of liver damage. The effect of feeding propylthiouracil on the other hand is not so clear cut. With a level of 0.3 per cent propylthiouracil the rats died at an average interval of 47 days but they showed no evidence of hepatic injury at death. It was assumed that the toxicity of the supplement

Apparently, Handler and Folli⁸ the time at which the rats died 0.5 per cent of propylthiouracil its effect of the animals to survive the

TABLE 3
COMPOSITION OF BASAL DIET

Dietary ingredient	Per cent
Yeast (Fleischmann's active dried baker's yeast)	18
Cornstarch	69
Lard	5
Salt mixture*	3
Sugar vitamin mixture**	1
Cod liver oil (Mead Johnson Company 3000 I U of Vitamin A and 400 I U of Vitamin D per gram)	2
Cellulose	2

* Ponsworth's salt mixture (Personal communication for details see Abell *et al.*)

** The sugar vitamin mixture contributed the following amounts of B vitamins per 3 gm of food: thiamin hydrochloride 20 µgm, riboflavin 25 µgm, pyridoxine hydrochloride 20 µgm, calcium pantothenate 100 µgm.

TABLE 4
THE EFFECT OF DIETARY SUPPLEMENTS ON THE DEVELOPMENT OF LIVER NECROSIS

Dietary supplement	Rats dying of necrosis or killed in extremis		Rats dying of other causes		Total incidence of necrosis, cord %	
	Number rats	Average days on diet	Number rats	Average days on diet	Number	%
None	9	39	1	42	9/10	90
0.3% desiccated thyroid	7	20	3	13	7/8	87.5
0.03% propylthiouracil	2	59	5	55	3/10**	30
0.3% potassium thiocyanate	8	56	2	69	8/10	80
0.3% desiccated thyroid + 0.3% L-cystine	0	—	8	57	0	0
0.3% desiccated thyroid + 2 mgm α-tocopherol acetate per 3 gm diet	0	—	9	43	0	0

antithyroid action of the drug, the possibility that the prophylactic action may be due to the provision of sulphhydryl groups must be kept in mind since György *et al.*¹¹ have shown that this compound exists in tautomeric forms.

According to Armstrong *et al.*¹² propylthiouracil may be considered to be a member of the latter group, and it was considered worthwhile to test the effect of a compound belonging to the former class. Potassium thiocyanate was chosen and, at a level of 0.3

per cent, effected a significant increase in the length of time required for death to occur, 56 days compared to 39 days for the rats on the basal diet. There was no effect, however, on the actual incidence of acute hepatic damage.

Supplementation of the basal diet with 0.5 per cent L-cystine or 2 mgm alpha tocopherol per 8 gm of diet did not overcome the well known toxic effects of excessive amounts of thyroid tissue, but the animals survived beyond the average time at which death occurred on the unsupplemented ration (indeed, three rats survived the experimental period of 105 days), and none of the rats in these groups developed massive hepatic necrosis. The fact that both alpha tocopherol and cystine prevented liver necrosis in hyperthyroid animals led us to conclude that a high metabolic rate *per se* is not a prime factor in pathogenesis and that the level of thyroid activity is a subsidiary, not a determining factor, in the production or prevention of this hepatic lesion.

From time to time, we have observed pancreatic necrosis in some of the rats killed in *extremis* and showing acute liver damage. This finding raised the possibility that, despite the absence of any consistent obvious lesion in the pancreas, this organ might be in some way involved in the process under study.

An initial pancreatic lesion might result in active proteolytic enzymes gaining the portal blood stream and causing the type of extensive liver damage under investigation here. Certainly, the intravenous administration of chymotrypsin as was shown by Tagnon *et al* causes wide spread damage including necrosis, not only of liver cells, but also of kidney tubules, a lesion that is also frequently seen in animals showing acute massive hepatic necrosis. The fact that the blood from the pancreas travels primarily to the left half of the liver fitted in quite nicely with the well substantiated observation that the left half of the liver is usually the most severely damaged part. It was decided therefore, to investigate the possible etiological role of the pancreas although it must be admitted that our thoughts were directed primarily towards the exocrine rat.

As complete a pancreatectomy as possible was performed on a number of males rats. Since a splenectomy was done in this procedure, control groups of rats were comprised not only of sham-operated animals, but also a group of which splenectomies had been carried out. These animals, of average initial weight about 140 gm were fed a basal necrogenic diet in the usual way and after an interval of 290 days the experiment was terminated. The results are shown in TABLE 5.

TABLE 5
EFFECT OF PARTIAL PANCREATECTOMY AND SPLENECTOMY ON THE DEVELOPMENT OF LIVER NECROSIS

Group	Number of rats showing liver necrosis	Incidence of necrosis	Days on diet of rat showing liver necrosis	
			Average	Range
Normal controls	1	1/11	68	143-213
Depancreatized and splenectomized	3	3/10	181	176-213
Splenectomized	3	3/11	195	176-213

Although we were somewhat disturbed by the low incidence of liver necrosis these findings show clearly that neither partial pancreatectomy plus splenectomy, nor splenectomy alone has any effect on the development of acute liver damage. On the other hand we readily admit that there may have been sufficient pancreatic tissue left to account for the production of the liver lesion, but we regard this possibility as being remote.

In conclusion it would appear that although various investigations have demonstrated that secretions from the endocrine glands do influence one way or another the development of dietary liver necrosis there is no clear cut evidence to show that any of the endocrine secretions have a primary role in the development of this hepatic lesion.

References

- 1 WEINSELBAUM T E 1935 Quart J Exptl Physiol 26 363
- 2 GYÖRGY P 1949 Abstracts 1st International Congress Biochem 90
- 3 ABELL M R & J M R BEVERIDGE 1951 Arch Path 61 423
- 4 FERRET P 1950 Brit Exptl Path 31 590
- 5 SCHWARZ A 1944 Z physiol Chem 281 101
- 6 SCHWARZ K 1951 Science 113 435
- 7 ABELL M R & J M R BEVERIDGE 1950 Arch Path 60 23
- 8 HANDLER P & R H FOLLIS 1948 J Nutrition 35 669
- 9 ABELL M R & J M R BEVERIDGE & J H FISHER 1950 Arch Path 60 1
- 10 McLEAN J R & J M R BEVERIDGE 1952 Can J Med Sci 30 118
- 11 GYÖRGY P & T STILLER & M B WILLIAMS 1943 Science 91 518
- 12 ASTROOD F B 1943 J Pharmacol Exptl Therap 78 79
- 13 TAGNON H J A R WEINGLASS & W E GOODPASTER 1945 Am J Physiol 143 644

Discussion of the Paper

DOCTOR KLAUS SCHWARZ I should like to point out that food intake greatly influences the development of dietary necrotic liver degeneration. This variable is of primary importance in the interpretation of results such as reported by Doctor Beveridge. We have investigated in collaboration with Doctor R. Scow of our Institute the effect of thyroidectomy on the development of this deficiency. It was found that Sprague Dawley rats when thyroidectomized at weaning did not develop necrotic liver degeneration even after experimental periods extended long beyond the time of death of all the littermate controls fed *ad libitum*. Realizing however that rats fed *ad libitum* do not constitute proper controls for others which do not eat due to some endocrine disturbance we have run simultaneous unoperated littermate controls which were paired to the thyroidectomized group. These animals which received approximately 50 per cent of the diet consumed by the *ad libitum* fed group survived as well as the thyroidectomized ones. It may be possible that we have to interpret all the reported effects of decreased or increased thyroid activity on necrotic liver degeneration as resulting from decreased or increased food consumption and not from the endocrine disturbance directly.

FACTORS PROTECTING AGAINST DIETARY NECROTIC LIVER DEGENERATION

By Klaus Schwarz

National Institute of Arthritis and Metabolic Diseases, National Institutes of Health
U S Public Health Service, Bethesda, Md

Introduction

It is a pertinent characteristic of every natural phenomenon that, in addition to its primary cause, secondary conditioning causes influence its origin. This is certainly true for all deficiency diseases. In the preceding papers, various nonspecific factors affecting the development of dietary necrotic liver degeneration in the rat have been presented and discussed. The present paper is devoted to the factors which are primarily involved.

The development of necrotic liver degeneration has been shown to depend on such complicating circumstances as the over all composition of the deficient diet, the endocrine status of the animal, its daily food allowance, and its environmental temperature. No matter how profoundly these various influences either enhance or inhibit the process the fact remains that dietary necrotic liver degeneration is, in the first place, a deficiency disease. It can be produced experimentally by diets which lack certain essential nutrients and it can be prevented by these dietary components. The disease is different, however, from many other deficiency diseases in that it does not result from the lack of one single dietary factor. It is more complicated. At least three different nutritional factors must be lacking simultaneously before the disease develops. This complexity has prevented a quick solution of the many problems which are presented by the deficiency. It also makes dietary necrotic liver degeneration especially interesting from a biochemical and metabolic point of view. Like fatty liver and cirrhosis, the other liver disease produced by dietary means, dietary necrotic liver degeneration is of multiple origin, but the nutritional factors involved are different. Substances which prevent the first syndrome can even enhance the development of the second, and vice versa.¹

The three nutritional factors at present clearly separable and recognized as protecting against dietary necrotic liver degeneration are cystine, vitamin E and Factor 3. The action of cystine was detected in 1935 by Weichselbaum and later by Daft, Sebrell and Lilhe,² it was confirmed by Hock and Fink³ and many others. The role of vitamin E as a protective agent was elucidated between 1941 and 1944 by Schwarz^{4, 5, 6} and has been confirmed by Gyorgy and collaborators⁷ and by various other groups between 1947 and 1950. Finally the existence of Factor 3 has been established in this laboratory only a few years ago.⁸ An account of this work is given below.

Diets for the Production of Dietary Necrotic Liver Degeneration

Rations designed to produce dietary necrotic liver degeneration must be low in cystine, free from vitamin E and, furthermore, deficient in Factor 3, since

* Printed and published in 1945 in the United States under the auspices of USOAPC by J W Edwards
Ann Arbor, Mich.

TABLE 1
DIETS PRODUCING NECROTIC LIVER DEGENERATION IN RATS

Cystine free (corn starch 80%, dry milk powder 16%)	Weichselbaum ²
Cystine free (amino acids in crystalline form)	DuVigneaud Dyer, and Kies ²⁴ Glynn Himsworth, and Neuberger ¹ Daft Sebrell and Lilhe ³ Gyorgy and Goldblatt ²⁵ Hove and Harris ²⁶ Schwarz ²
Low casein (10% or less)	Hock and Fink ⁴ Glynn and Himsworth ¹⁵ Schwarz ² 25 Gyorgy and Goldblatt ²⁶ Schwarz ²⁷
Casein VI (alkali treated, 15%)	
Yeast	
High cod liver oil	
Potato protein	in various combinations
Pea protein	
Cereal protein	
Gelatin	
Soybean meal (raw, fat extracted)	
Soybean protein	Hock and Fink ²⁸
	Matet Matet and Fridenson ²⁹ Olson ³⁰

TABLE 2
COMPOSITION OF DIETS

Casein VI diet		Torula yeast diet	
	Parts		%
Casein VI*	15	Torula yeast	30
Butter fat (extracted)*	5**	Lard, Vit E-free††	5
Sucrose	65	Sucrose	59
Salts***	5	Salts†	5
Vitamins supplemented separately†		Vitamin powder††	1

* Preparation described in Schwarz²

** In earlier experiments 10 parts

*** For composition see Schwarz²

† See Schwarz²

†† Vitamin E free animal fat stripped by molecular distillation (D distillation Products Division, Rochester, New York)

‡ Composition same as salts in Casein VI diet since April 1951 Hubbel Mendel and Wakeman J. Nutrition 14: 273 (1937)

††† Composition see Schwarz²

sulfur amino acids in respect to dietary necrotic liver degeneration. It is generally accepted that methionine and not cystine is the sulfur-containing amino acid essential for growth and for well being of animals. It is quite evident that the rat forms cystine from methionine, whereas it is unable to form methionine from cystine. The requirement for sulfur amino acids can be satisfied by methionine alone but not by cystine¹⁴. In dietary necrotic liver degeneration, on the other hand we have a case where cystine is more important than methionine, at least in the survival of the animal. Cystine affords protection, whereas methionine is much less effective. This was discovered in 1935 by Weichselbaum² and was later demonstrated by Himsworth and collaborators, who at first considered necrotic liver degeneration to be a methionine deficiency¹⁵ and then upon more careful investigation, asserted that

TABLE 3

COMPARISON OF PROTECTIVE EFFECTS OF L-CYSTINE AND L-METHIONINE AGAINST DIETARY NECROTIC LIVER DEGENERATION

Supplement	%	% protection*
L-cystine	0.1	17
L-methionine	0.123	3
L-cystine	0.2	68
L-methionine	0.240	12

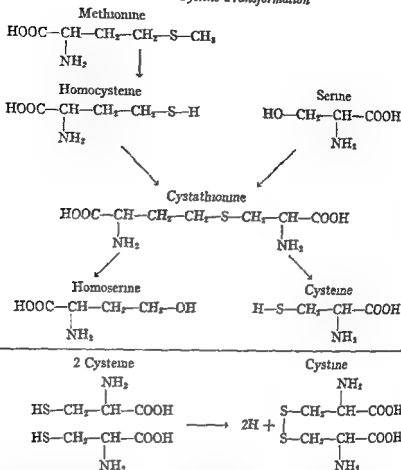
* Per cent protection = per cent reduction of necrotic area val time as compared to littermate control group

not methionine, but rather cystine, is the protective agent¹⁸. When L-cystine and L-methionine are compared at different dose levels in the Torula diet it is seen that supplements of L-methionine are not entirely ineffective. They show usually about 25-35 per cent of the activity of equivalent amounts of L-cystine. A representative experiment is given in TABLE 3.

Teleologically, it could be theorized from these results that in an animal succumbing to liver necrosis, the transformation of methionine to cystine is not sufficient to prevent death. If this were a metabolic insufficiency it could be due either to an increased demand for cystine exceeding the natural tendency and capacity to perform this conversion or to a real inhibition of the transformation by a metabolic block which could be caused by lack of vitamin E and Factor 3. It is conceivable, but requires further elucidation that the methionine-cystine transformation occupies a key position in the antagonistic relationship between the factors which protect against dietary necrotic liver degeneration (prevented by cystine) on the one hand and the factors which protect against fatty liver and cirrhosis (prevented by methyl groups) on the other. The pathway of the transformation as it is understood at present involves the main reactions found on the following page.

In an attempt to detect which step of the transformation is inadequately performed, various intermediates were fed. DL homocysteine was as ineffective as methionine. Cystathionine was not tested. The last step in the conversion of methionine to cystine is the oxidation of cysteine to cystine. It was found, to our surprise, in repeated experiments that L-cysteine when fed either by stomach tube or when given in the diet does not replace L-cystine. The activity of cysteine is as low as that of methionine (TABLE 4).

From these results a number of essential questions can be derived. One might ask whether cysteine is as easily converted to cystine in normal intermediary metabolism, as is usually taken for granted or whether in animals on necrosis producing diets this oxidative process is especially defective. The question also arises whether vitamin E or Factor 3 participates catalytically in this reaction or in some other closely associated reaction of sulfur amino acid metabolism. Furthermore, it becomes a question as to whether the effect of cystine on dietary necrotic liver degeneration is strictly a physiological nutritional one or whether cystine in this instance exerts some "pharmacological" action in excess of its normal biological functions and in excess of the normal cystine requirement. It deserves attention that the effect of cystine

Methionine Cystine Transformation

is obviously not due to a known detoxification mechanism for the simple reason that in detoxification reactions cysteine is as active as cystine¹⁷

Vitamin E as a Protecting Agent

After two years highly purified fractions were obtained. The best concentrates contained up to 44 per cent of a substance which was shown to be vitamin E. Early in 1943 synthetic α tocopherol acetate was found to be active.

From a series of tests performed in 1943 the daily dose of synthetic α tocopherol acetate required for 50 per cent protection was found to be 67 γ . Using

TABLE 4
COMPARISON OF L-CYSTINE AND L-CYSTEINE (SUPPLEMENTATION STARTED ON THE 23TH DAY)

Supplement	%	% protect on*
L-cystine	0.5	96.6
L-cystine	0.5	16.5
L-cystine	1.0	97
L-cystine	1.0	26.2

* Per cent protect on = per cent reduction of necrotic survival time as compared to termate control group

the Torula yeast diet and Sprague Dawley rats in Bethesda in 1950 we found that about 50 γ are required daily for the same effect. In the later experiments the vitamin was added to the diet. These levels of vitamin E required were in the earlier publications considered to be much higher than the normal vitamin E requirement. Dietary necrotic liver degeneration was interpreted as a 'relative' vitamin E deficiency.⁶ In view of more recent data, however, it appears that these quantities of vitamin E are actually well within the range of the normal vitamin E requirement for the prevention of deficiency symptoms in rats. It has, for example, been reported that between 30 and 70 γ of synthetic α -tocopherol acetate are required daily for maintenance of normal seminiferous epithelium in growing rats.¹² In the light of these observations dietary necrotic liver degeneration appears to be a form of vitamin E deficiency which is modified by the simultaneous absence of Factor 3. The occurrence of dietary necrotic liver degeneration as a vitamin E deficiency indicates strongly that tocopherol has functions which are of basic importance for normal intermediary metabolism.

Factor 3 against Dietary Necrotic Liver Degeneration

An attempt was made in 1943-1944 to determine whether the procedure used for the preparation of casein VI from Hammarsten casein caused a toxic component to be formed or whether a protective factor was removed. Evidence for the first possibility could not be found and some evidence for the later was obtained. Since Hammarsten casein from which the casein VI was produced, is suitable for the production of vitamin E deficiencies the protective substance removed could be assumed to be different from tocopherol. Analyses for cystine and methionine showed that the difference between the two caseins could not be ascribed to differences in cystine or methionine (TABLE 5).

The reasoning which led to the discovery of Factor 3 may be further illustrated by the following example. Two diets are listed in TABLE 6. One is a conventional 15 per cent 'vitamin free' casein diet, the other a 30 per cent Torula ration (approximately 14 per cent protein). The first does not produce dietary liver necrosis. The other one prepared with the same dietary ingredients except for the substitution of Torula yeast at the expense of casein and of sucrose, produces the damage with 100 per cent incidence. When the ingredients used in this experiment were analyzed colorimetrically and microbiologically for cystine and methionine, it became evident that there was no profound

TABLE 5
S-AMINO ACIDS IN CASEIN DIETS

	Cystine	Methionine	Incidence of dietary necrosis liver degeneration
	%	%	%
Casein Hammarsten diet (15%)	0.06	0.52	0
Casein VI diet (15%)	0.00	0.54	84

TABLE 6
S-AMINO ACIDS IN TORULA DIET

	Cystine	Methionine	Incidence of dietary necrosis liver degeneration
	%	%	%
Control diet, 'Vitamin free' casein (15%)	0.075	0.45	0
Torula yeast diet (30%)	0.29	0.38	100

and decisive difference in sulfur-containing amino acids. The Torula diet was even somewhat superior in cystine, but the differences were altogether so small that they were, in view of the relatively high levels of cystine and methionine required for protection, not of significance. Accordingly, the fundamental dissimilarity between these two rations in regard to liver necrosis could not be attributed to differences in cystine or methionine. Moreover, the dissimilarity could not be caused by a difference in the vitamin E contents, since both rations were apparently practically free from tocopherol. Both "vitamin free" casein, on the one hand, and dried yeast, on the other, are standard ingredients of diets producing vitamin E deficiencies of the conventional type. From these considerations, it became evident that the difference between the two diets could be expected to be due to some other factor, and it seemed logical to test the "vitamin free" casein for liver protecting activity in the Torula assay (FIGURE 1). Addition of 3 per cent of the "vitamin free" casein produced a reduction of 66 per cent in reciprocal survival time. By comparison, an amino acid mixture simulating the composition of the casein¹⁸ was without significant effect.

The detection of Factor 3 was facilitated by a peculiar discrepancy between European and American yeasts. In vitamin E-free diets, almost all European dried yeasts led to the deficiency²⁰ whereas almost all American yeasts, particularly American brewers' yeasts, did not produce the disease.^{9, 21} However, an American Torula yeast which is grown commercially on a rather simple medium was found, in 1949, to produce the deficiency with 100 per cent incidence.⁹ Since that time the 30 per cent Torula diet has been used in our laboratory in extensive investigations in attempts to concentrate and identify Factor 3.

TABLE 7

So far, no
offer protection

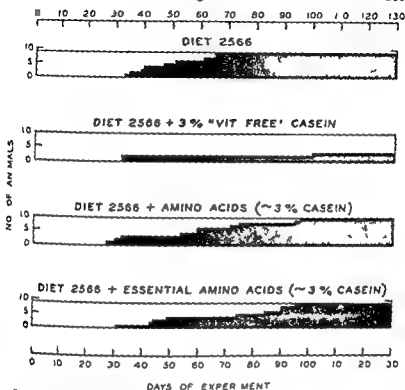


FIGURE 1. Prevention of death from dietary liver degeneration by 3 percent casein (Black arrows: Animals dead on account of liver degeneration).

why the disease could not be produced in numerous attempts with diets based mainly on these yeasts.

As starting material for the purification for Factor 3 a variety of natural sources can be used. Factor 3 has in common with most other essential accessory foodstuffs, the property of being strongly bound to proteins. It cannot be liberated by ordinary extraction procedures. Therefore most of the sources of Factor 3 have to be acid hydrolyzed before the fractionation of the active principle can begin. Acid hydrolysis does not alter the total Factor 3 activity since the active substance is quite stable against this treatment. Factor 3 is found in free form in enzymatic protein digests. A commercial casein preparation has been used for the large scale purification of the active substance. The first three steps of the purification have been described elsewhere.¹ The active

TABLE 7
PROTECTIVE EFFECT OF YEAST K

Torula yeast %	Supplements	No. of A animals	Dead due to liver degeneration	Avg. of reciprocal survival time (X 100)	% protection*
30	—	9	9	240 ± 18	—
27	Yeast K 3%	10	2	58 ± 25	76
25	Yeast K 5%	8	0	100 ± 0	100

* Per cent protection = per cent reduction of reciprocal survival time as compared to littermate control group

a small volume of water. From this fraction, a heavy precipitate is formed upon standing in the cold, which contains most of the Factor 3 activity. Further steps of purification involve treatment with solvents like ethanol, butanol, and phenol, and also precipitation reactions with phosphotungstic acid and barium hydroxide. In this manner, fractions can be obtained from casein which protect the animals from dietary necrotic liver degeneration when given in very small amounts.

since the customary reactions known to destroy these substances do not inactivate our fractions. Factor 3 can be expected to have a rather low molecular weight. It might well be that it is identical with a compound which is already known, though a large number of substances occurring in nature have been tested and found to be inactive.

Metabolic Interrelations in Dietary Necrotic Liver Degeneration

The peculiar fact that, in dietary necrotic liver degeneration, three sub-

and Factor 3, on the other hand, may be remains to be clarified. The quantitative proportions between these substances have to be elaborated when all of them are available in pure form. Considering the fact, however, that between 400 to 800 molecules of cystine are required to produce as much protection as that offered by one molecule of α -tocopherol, it could be postulated that the role of vitamin E is of a catalytic nature whereas cystine or a cystine derivative functions as a substrate.²² From the dosage levels of Factor 3 required to obtain comparable degrees of protection, it can be suggested that all these substances belong to the group of substances which have catalytic

the dietary relationship described

An attempt has been made to

of the disease, i.e.

In preliminary

ner of animals on

injection of Na monofluoroacetate. For weeks prior to succumbing to liver necrosis, animals on necrogenic diets show a serious incompatibility for fat. These and other findings make it conceivable that the area of the primary metabolic breakdown in dietary necrotic liver degeneration is in the citric acid cycle or in some closely related function.

Summary

Three different nutritional factors have been recognized to be specifically connected with dietary necrotic liver degeneration: cystine, vitamin E, and Factor 3. Diets for the production of the deficiency in rats must be low in cystine and deficient in vitamin E and in Factor 3. Two rations fulfilling these requirements are described: the casein VI diet, used between 1940-1943, and the 30 per cent Torula yeast ration, in use since 1949.

In the prevention of dietary necrotic liver degeneration, cystine is required in rather large amounts (0.2-1 per cent in the diet). Other S-containing amino acids, like methionine, homocystine and also cysteine are only approximately one third as effective as cystine.

α -Tocopherol acetate was found to afford 50 per cent protection at 67 γ daily per animal in the old series and about 50 γ daily in the new series. These levels are within the range of the normal vitamin E requirement of the rat.

The detection of Factor 3, its occurrence in various caseins and in brewers' yeast, and the method of purification of Factor 3 concentrates are described. Factor 3 is of low molecular weight, stable against acid hydrolysis, water soluble, and apparently not identical with any of the presently well-known vitamins or amino acids.

The possible metabolic interrelations between these factors and also the metabolic aspects of dietary necrotic liver degeneration are discussed. Preliminary results of an elimination study in the liver are mentioned.

Primary metabolic defects

References

- SCHWARZ, K. 1953. Introduction. Liver Necrosis Versus Fatty Liver and Carcinoma. N. Y. Acad. Sci. 57(6).
 REICHELBAUM, T. E. 1935. Quart. J. Exptl. Physiol. 25: 363.
 DART, F. S., W. H. SERRILL & A. D. LILLIE. 1942. Federation Proc. 1: 159.
 Proc. Soc. Exptl. Biol. Med. 50: 1.
 HOCK, A. & H. FINK. 1943. Z. physiol. Chem. 278: 136.
 SCHWARZ, K. 1944. III. physiol. Chem. 281: 101.
 SCHWARZ, K. 1944. Z. physiol. Chem. 281: 109.

ENZYME ABNORMALITIES ASSOCIATED WITH DIETARY NECROTIC LIVER DEGENERATION IN RATS*

By Robert E. Olson and James S. Dinning

*Department of Biochemistry and Nutrition, Graduate School of Public Health,
University of Pittsburgh, Pittsburgh, Pa.*

Although acute massive hepatic necrosis in the rat has been shown to be a manifestation of malnutrition, its exact biochemical pathogenesis is unknown. Frank tissue necrosis is generally assumed to be preceded by single or multiple biochemical lesions, and it seems probable that this particular syndrome of hepatic necrosis is no exception to the rule. The fact that a deficiency of one or more dietary nutrients appears to be related in a causal manner to this syndrome was of particular interest to us and led us to undertake a study of its biochemical evolution. It was hoped, in addition, that these studies would contribute to the elucidation of the interrelationships between the sulfur amino acids and vitamin E, both of which are protective against this disease. Our initial approach to this problem was to compare the content of selected enzymes and coenzymes in the livers of rats in which hepatic necrosis was produced with others which were protected by suitable dietary supplements. The results, though preliminary, are consistent with the view that acute massive hepatic necrosis in the rat is associated with differential disruption of the citric acid cycle and depressed tissue levels of coenzyme A.

Male weanling rats of the Sprague Dawley strain were used in these experiments. One group was fed the basal diet composed basically of soy protein and lard.

TABLE I
devoid of
plus pro

protective supplements. These included Vitamin E alone, cystine alone, cystine plus Vitamin E, methionine alone, and methionine plus vitamin E. Vitamin E was administered at the level of 3 mgm. of α -tocopherol twice weekly by gavage and the basal diet contained a level of 0.5 per cent.

lated intermediate growth rates between that observed on the basal diets and the diet containing 18 per cent casein.

After 4 to 6 weeks, animals on the basal diet developed lesions typical of acute massive hepatic necrosis both grossly and microscopically. No lesions were observed in the rats receiving vitamin E or methionine. Occasional microscopic lesions were observed in the rats receiving the basal diet.

At the time of the first deaths in the basal group, rats were taken from all groups for the determination of hepatic pyruvic oxidase, succinioxidase, trans-

* Supported in part by grants in aid from the Nutrition Foundation, Inc., New York, N. Y., and the Williams-Waterman Fund of the Research Corporation, New York, N. Y.

TABLE 1
BASAL DIET

	Per cent
Alpha protein	15.0
Sucrose	72.8
Lard	6.0
Salts (H M W)	4.0
Cod liver oil	2.0
Choline	0.2

B vitamins were added in the following amounts expressed as mgm. per kgm. diet: Thiamine 5.0, riboflavin 5.0, pyridoxine 5.0, calcium pantothenate 20.0, niacin 40.0, folic acid 1.5, and biotin 0.05. Menadione was added at the level of 0.08 mgm./kgm.

GROWTH IN RATS

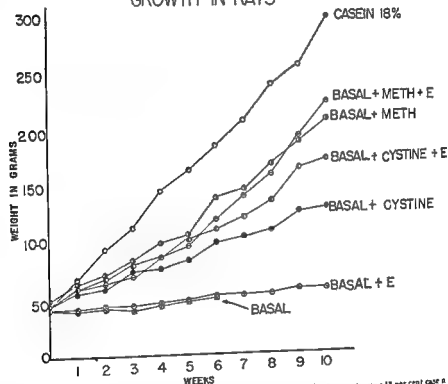


FIGURE 1. Growth rates of weanling rats fed the experimental diets and a diet containing 18 per cent casein over a 10-week period.

aminase and xanthine oxidase. These particular enzymes were chosen for assay in order to gain as much information as possible about the carbohydrate amino acid and purine metabolism of necrotic and protected livers. The animals were killed by decapitation and 10 per cent homogenates of liver in isotonic KCl were prepared. Appropriate amounts of homogenate were taken for each of the assays.

The term pyruvic oxidase is used in this paper in its broadest sense of includ

TABLE 2

PYRUVATE OXIDATION IN HOMOGENATES OF LIVER FROM NECROTIC NON NECROTIC DEFICIENT VITAMIN E- AND SULFUR AMINO ACID-TREATED RATS

Diet	Rats nr	Necrosis gross	QO ₂ none	QO ₂ pyruvate	QO ₂ pyruvate + ATP
Basal	9	+	1.0 ± 0.1	1.5 ± 0.3	3.1 ± 0.6
Basal	5	■	1.2 ± 0.6	2.8 ± 0.4	7.2 ± 0.9
Basal + E	■	0	2.9 ± 0.6	3.2 ± 0.8	6.9 ± 0.9
Basal + cystine	5	■	3.2 ± 0.7	4.8 ± 0.5	6.0 ± 0.9
Basal + cystine + E	5	0	4.0 ± 0.4	5.7 ± 0.7	8.9 ± 1.3
Basal + meth.	4	0	3.3 ± 1.0	3.2 ± 1.1	7.9 ± 0.8
Basal + meth. + E	5	0	3.1 ± 0.4	4.2 ± 0.4	8.8 ± 0.8

... which substitute with sodium pyruvate added at 5 mM/Liter, and with pyruvate at 5 mM/L, plus ATP at 1 mM/L. The addition of ATP to the last flask permitted the observation of pyruvate oxidation under conditions which tended to prevent deactivation of tricarboxylic acid cycle intermediates and the fission of coenzymes. Succinioxidase was determined by the method of Schneider and Potter² transaminase by the method of Ames and Elvehjem⁶ and xanthine oxidase by the method of Axelrod and Elvehjem.⁷ All substrates were tipped in at "zero" time. The units for the pyruvic oxidase assay are given as QO₂, calculated for a 20-minute period and the units for the other assays are those used by their respective authors.

The results obtained with the pyruvic oxidase assay are shown in TABLE 2. The animals on the basal diet were divided into two groups depending upon the presence or absence of gross necrosis. It can be seen that both the endogenous O₂ uptake and the oxidation of pyruvate, with or without added ATP was markedly reduced in homogenates of liver from rats showing gross necrosis. The activity of the control group (rats fed basal plus methionine plus vitamin E) ...

... groups fed the basal diet plus vitamin E were intermediate between the rats on the basal diet not showing necrosis and the controls although not significantly different from either. None of the rats on other protective regimens showed values significantly different from the controls.

TABLE 3 presents the results of the assays for succinioxidase, transaminase and xanthine oxidase in these same groups of animals. It is noteworthy that the activity of succinioxidase and transaminase are virtually the same in all groups. The ...

... statistically ... ent from the ... in comparison with the drop in pyruvic oxidase activity. It seemed remarkable to us that hepatic transaminase activity was maintained in the severely

TABLE 3
SUCCINOXIDASE TRANSAMINASE, AND XANTHINE OXIDASE ACTIVITIES OF LIVER FROM
NECROTIC, NON-NECROTIC-DEFICIENT, VITAMIN E- AND SULFUR AMINO
ACID-TREATED-RATS

Diet	Rats no	Necrosis gross	Succinoxidase	Trans-aminase	Xanthine oxidase
Basal	9	+	31 ± 3	64 ± 2	39 ± 9
Basal	5	0	38 ± 4	55 ± 5	13 ± 9
Basal + E	6	0	39 ± 3	60 ± 4	6 ± 5
Basal + cystine	5	0	39 ± 2	57 ± 4	137 ± 8
Basal + cystine + F	5	0	47 ± 5	65 ± 9	20 ± 13
Basal + meth	4	0	40 ± 2	63 ± 11	160 ± 10
Basal + meth + E	5	0	42 ± 2	62 ± 8	138 ± 8

necrotic group, particularly since it has been reported that certain manifestations of pyridoxine deficiency appear in association with vitamin E deficiency in rabbits.¹⁷

The xanthine oxidase activity of the livers from animals in this study were of great interest. The lowest values were obtained in rats fed the basal diet plus vitamin E. This was probably due to the poor biological value of the

oxidase. This effect of vitamin E lack mirrors an effect seen in rabbits deprived of vitamin E, in which the hepatic xanthine oxidase becomes markedly elevated.⁹ The tendency of vitamin E to depress the liver xanthine oxidase is also demonstrated by a comparison of the liver xanthine oxidase values of the groups given cystine alone and cystine plus vitamin E (TABLE 3). When methionine is the supplement, the vitamin E effect is abolished. Whether this vitamin E effect is dependent upon adequate amounts of molybdenum (which is abundant in soy protein) in the diet,^{10, 11} remains to be determined. If methionine is required for the elaboration of xanthine oxidase in the liver these effects of vitamin E might be explained on the basis of a decreased utilization of methionine for other purposes in vitamin F deficiency. The effect of cystine would be interpreted as a sparing action.

decline of the activity of all the cellular enzymes, in addition to catabolic ones known to increase with cell lysis such as the cathepsins, desamidases, phosphatases, and nucleases, may even increase. How much of this change can be traced to the underlying pathological process preceding necrosis and how much is a concomitant of necrosis *per se*? The disruption of some portion of the citric acid cycle exclusive of the succinoxidase system seemed out of proportion to the other changes and it was thought profitable to explore the status of certain catalysts in that sequence of reactions. There were obviously many possibilities. The observation that the

TABLE 4

CONTENT OF COENZYME A IN LIVER FROM NECROTIC NON NECROTIC-DEFICIENT VITAMIN E TREATED, AND CONTROL RATS

Diet	Rats per	Necrosis gross	Coenzyme A units/gm
Basal	9	+	37 \pm 5
Basal	5	0	68 \pm 9
Basal + E	4	0	92 \pm 8
Control	4	0	180 \pm 20

rats on the basal diet developed the "porphyrin whiskers" usually associated with pantothenic acid deficiency led us to determine the rates of acetylation of para aminobenzoic acid in intact deficient rats. The finding that there were markedly depressed in the animals with liver necrosis and moderately depressed in deficient animals without necrosis¹² coupled with the realization that coenzyme A, the active form of pantothenic acid in tissues and the bearer of the α -fragment, contained a sulfur moiety¹³ not unrelated to cystine, stimulated us to make direct determinations of coenzyme A in livers from these animals. The technique of assay was that of Kaplan and Lipmann¹⁴ and the results are shown in TABLE 4.

The rats having liver necrosis had markedly depressed values for hepatic coenzyme A. Those on the deficient diet not showing necrosis had levels somewhat higher, but even these were as low as ordinarily produced by pantothenic acid restriction alone.¹⁵ Those rats protected against necrosis with vitamin E had still higher values and control rats fed either chow or the 10 per cent casein diet had high values which were similar to other normal rats examined in the past. Because of this relationship between coenzyme A content and the suspected degree of hepatic disability in this dietary disease,

hypothesis

Is the depression of coenzyme A in necrotic liver tissue specific for this dietary deficiency disease, or does it represent a generalized result of necrosis? The fact that transaminase and xanthine oxidase activities which are also dependent upon coenzymes (pyridoxal phosphate and flavin adenine dinucleotide) are well maintained, and the fact of the low coenzyme A content of non necrotic deficient liver tissue suggest that the coenzyme depletion of necrotic liver tissue is not nonspecific. It will be important however, to make direct determination of other coenzymes in the livers of deficient rats and these studies are in progress.

Is β mercaptoethylamine, the sulfur moiety of coenzyme A derived from dietary cystine? One can visualize a reasonably simple pathway from cystine to β -mercaptoethylamine in one step of reduction and one step of decarboxylation although the order of the steps and all of the enzymes involved are not known. Studies with S^{35} labeled cystine are in progress to verify this relationship and a search for the probable enzymes involved is under way.

Finally, the possible role in hepatic necrosis of another sulfur containing coenzyme in the metabolism of pyruvate α lipoic acid must be ruled out¹⁶. Although this coenzyme is normally synthesized by mammalian tissues it is conceivable that rigid organic sulfur restriction in the diet may also limit its synthesis.

The conclusions which can be drawn from this preliminary study are necessarily subject to qualification. I believe it can be said however that rat liver rendered necrotic by dietary restriction of cystine methionine and vitamin E presents an enzyme profile which features a defect in the oxidation of pyruvate which does not involve all enzymes of the citric acid-cycle. It may be said furthermore that dietary restriction of the sulfur amino acids results in a lowering of coenzyme A equivalent to that seen in marked pantothenic acid deficiency prior to the onset of hepatic necrosis and suggests that a nonvitamin precursor of a tissue coenzyme may be as important in the elaboration of the coenzyme as the vitamin itself. Vitamin E protects against hepatic necrosis tends to preserve the coenzyme A level and appears to divert dietary methionine into channels other than the synthesis of xanthine oxidase.

Acknowledgments

It is a pleasure to acknowledge the technical assistance in these experiments of Suzanne Maury Jean Sturm Marcia Riegl Ruth Neatroux and Richard Shaw. The authors are also indebted to Dr Frank Sherman of the Department of Pathology of the University of Pittsburgh School of Medicine for making the pathological observations.

References

1. HENSWORTH H. P. 1947. *The Liver and Its Diseases*. Harvard Univ Press. Cambridge Mass.
2. OLSON R. E. 1951. *Cancer Research* 11: 571.
3. SCHNEIDER W. C. & V. R. POTTER. 1943. *J Biol Chem* 149: 217.
4. AMES B. R. & C. A. ELVEHJEM. 1946. *J Biol Chem* 166: 81.
5. AXELROD A. E. & C. A. ELVEHJEM. 1941. *J Biol Chem* 140: 725.
6. BARBER M. A. D. H. BASINSKI & R. A. MATILL. 1949. *J Biol Chem* 181: 17.
7. DINNING J. S. 1953. *Federation Proc* 12: 412.
8. LITWACK G. J. N. WILLIAMS L. CHEN & C. A. ELVEHJEM. 1952. *J Nutrition* 47: 299.
9. DINNING J. S. 1953. *J Biol Chem* 202: 213.
10. WESTERFELD W. W. 1953. The Xanthine Oxidase Factor (Molybdenum). *Ann N Y Acad Sci* 57(6).
11. DE RENZO E. C. E. KALLITO P. HEYTLER J. J. OLSON B. L. HUTCHINGS & J. H. WILLIAMS. 1953. *J Am Chem Soc* 75: 753.
12. OLSON R. E. & J. D. MINYORD. Unpublished data.
13. NOVELLI G. O. J. D. GREGORY R. M. FLYNN & F. J. SCHMETZ. 1951. *Federation Proc* 10: 229.
14. KAPLAN N. O. & F. LIPMAN. 1948. *J Biol Chem* 174: 37.
15. OLSON R. E. & N. O. KAPLAN. 1948. *J Biol Chem* 175: 115.
16. REED L. J. & M. DEBUSK. 1952. *J Am Chem Soc* 74: 3964.

Discussion of the Paper

DOCTOR KLAUS SCHWARZ. The results of Doctor Olson and his collaborators are in harmony with some of our own. Investigations in our laboratory have shown that the site of the primary metabolic defect in the dietary necrotic

liver degeneration*

panethine (*Lactobacillus bul*
activity against dietary nec
without effect under our ex

References to the Discussion

- 1 Presented at the Gordon Research Conference New London N. H. Aug 10 1940
- 2 SCHWARTZ ■ 1953 Factors protecting against dietary necrotic liver degeneration
Ann N Y Acad Sci 57(6) 878

THE XANTHINE OXIDASE FACTOR (MOLYBDENUM)*

By W W Westerfeld and Dan A Richert

Department of Biochemistry, Medical College of State University of New York, Syracuse N Y

The effect of diet on the xanthine oxidase activities of various rat tissues was studied as a sequel to an earlier investigation in which it was observed that

systems that could theoretically be concerned with acetaldehyde metabolism, the chemically similar xanthine and aldehyde oxidases seemed most likely to be dependent upon a dietary factor not already incorporated in the purified rations used. Since methods for studying xanthine oxidase were available, our attention was directed to a study of the relationship between tissue xanthine oxidase in rats and the dietary factors supplied to the animals.

Xanthine oxidase in rat tissues At the time this work was started, riboflavin¹ and protein deficiencies² had been reported to decrease the concentration of liver xanthine oxidase in the rat. Our early studies were directed toward determining (1) the distribution of xanthine oxidase in rat tissues and (2) the

or similar to that shown in TABLE 1. Low protein diets were prepared by replacing all or part of the casein with additional glucose.

The results of these studies⁴ with adult rats are shown in TABLE 2. Small amounts of the enzyme were found in lung, kidney, spleen, and skin, but only

amount of enzyme was present in the liver while 20 per cent was found in skin and small intestine. Feeding a low protein diet removed at least 70 per cent of the total enzyme from the rat, but the amount remaining was adequate to maintain a normal excretion of uric acid and allantoin.⁶ This deserves some emphasis because it indicates that either (1) uric acid can be formed by a mechanism not involving xanthine oxidase or (2) the amount of xanthine oxidase normally present in the rat far exceeds the amount required to fulfill its usual function. *In vitro* studies⁶ could not detect any metabolic pathway for the formation of uric acid other than the one utilizing xanthine oxidase. The remaining conclusion is that rat tissues normally have at least a three fold

* The studies reported herein were supported by grants from the Nutriton Foundation, the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council, and the Office of Naval Research.

TABLE 1
COMPOSITION OF THE PURIFIED DIET USED IN STUDIES OF THE XANTHINE OXIDASE FACTOR

	gm	mgm
Casein (Labco)	100	100
Crisco	4	2.4
Wesson oil	2	1.0
Cod liver oil	1	0.4
Salts (Phillips and Hart)*	4	0.4
Glucose	65	0.4
		Pyridoxine

* P & H salts contain Na K Ca Mg Fe Mn Zn Cu Cl F phosphate sulfate carbonate and citrate

TABLE 2
THE EFFECT OF FEEDING A PURIFIED 8 PER CENT CASEIN DIET ON THE XANTHINE OXIDASE ACTIVITIES OF ADULT RAT TISSUES

	Liver	Small Int.	Kidney	Lung	Spleen	Heart
Normal rats (on chow)	41	33	4	9	12	4
8% casein diet (4-23 weeks)	2*	6*	5	8	14	—
Normal (6%) distribution	55	20	1	1	2	21

XO activities recorded as c.m.u. per 20 minutes per 100 mgm. fresh tissue. At least 70 per cent of XO activity in rat is lost on a low protein diet.

* The usual determination gives values of 0 and 2 for liver and intestine respectively. The recorded values represent the "true" amount present in relation to the normal values (see section on determination of xanthine oxidase).

reserve of this enzyme. Variations in tissue enzyme concentration or activity cannot automatically be interpreted as a corresponding variation in function.

Liver and intestinal xanthine oxidase. The weanling rat responded to low protein and other dietary changes more rapidly than the adult rat but other

containing adequate protein and riboflavin allowed the enzyme to return to intermediate levels, but did not permit the attainment of normal values. The incorporation of liver in a diet containing inadequate protein did not modify the protein deficiency depletion of liver xanthine oxidase. When the livers were first depleted of xanthine oxidase by a low protein diet the resumption of an adequate protein intake also restored the liver xanthine oxidase to about 75 per cent of the normal level.

It was concluded that the liver xanthine oxidase concentration depended

could not be identified with any known nutritional substance, although it was the liver residue factor or the xanthine oxidase factor (XOF). The magnitude of the effect of the xanthine oxidase factor on the concentration of liver xanthine oxidase was not large, but it was detectable and measurable.

Numerous successful tests were conducted, using the liver xanthine oxidase response as the criterion for the presence of this unidentified factor in the diet, but it was a difficult test to use as a routine assay procedure.

The source of XOF for the 75 per cent restoration of liver xanthine oxidase on a purified diet was unknown. In retrospect, it may have been derived from the bones. The relatively small effect of an XOF deficiency on liver XO could also be due to the availability of bone molybdenum for this response. The "dominant" effect of protein in controlling the level of liver xanthine oxidase may be an artifact because it might not be possible to produce a severe deficiency of molybdenum in the liver so long as preformed deposits exist in bones.

The effect of protein deficiency on other selected liver constituents has also

xanthine oxidase at weaning by virtue of its previous milk diet. Feeding a purified diet which was deficient in either protein or the xanthine oxidase factor were required simultaneously for this response, and protein was not a dominant factor in the intestinal response as it was for liver XO.¹⁴ With adequate protein in the diet, intestinal xanthine oxidase reflected the XOF content of the diet, and a bioassay procedure for XOF was established on this basis.¹¹

Determination of xanthine oxidase. Since all of these results depended upon the determination of xanthine oxidase in rat tissues, the accuracy of the method used in determining this enzyme was studied. The results in this and other papers were obtained by the manometric procedure originally described by Axelrod and Elvehjem.² correct values for liver

made by determining the activity in the pure activity could be determined in the usual manner and comparative relationships then calculated from the known and constant methylene blue relationship.^{10, 13} In contrast to an homogenate, xanthine oxidase appeared to be relatively inert in a slice.¹²

columns labeled and XOF deficiencies in liver dled "detn" show the relationship calculated from the values obtained in the presence of methylene blue. The true relationship shows

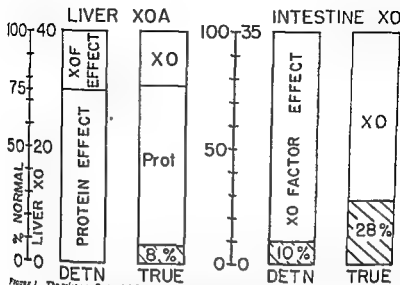


FIGURE 1. The relative effects of deficiencies of protein and xanthine oxidase factor on liver and intestinal xanthine oxidase. The XO factor effect is obtained in the presence of a separate protein. Normal values for liver and intestinal XO were chosen as 40 and 35 (c. mm. O₂ /20 min./283 mgm. fresh tissue) respectively. Cross-hatched areas are residual enzyme not removed by dietary deficiencies.

the presence of residual enzyme which cannot be removed by dietary means. The magnitude of the XOF effect (in the presence of adequate protein) is obviously much greater for intestine than liver.

Xanthine oxidase is not the same enzyme in all species. The enzyme in rat liver could be shown to have separate dehydrogenase and oxidase activities by the use of antabuse as an inhibitor of the oxidase portion of the enzyme¹⁰. The enzyme present in bird tissues was identified as a dehydrogenase rather than an oxidase^{11, 12} and the effect of dietary variations was shown¹³ to be somewhat different for the dehydrogenase in bird tissues as compared with the oxidase in mammalian tissues.

Biochemistry of Xanthine Oxidase

Cultured cells, casein diets, liver tissue, and published assay curve proved very satisfactory. However, the control levels of intestinal XO increased to 12 or 15 and occasional groups reached 20. Assay curves with such different batches of GBI casein are shown in FIGURE 2. The narrowing of the assay range and the increasing difficulty of the assay procedure as the control level of intestinal XO increased is evident. Since it is known that the hot alcohol extraction used for the purification of GBI casein does not remove XOF, the differences recorded in FIGURE 2 are dependent upon chance variation in the purity of the original untreated casein.

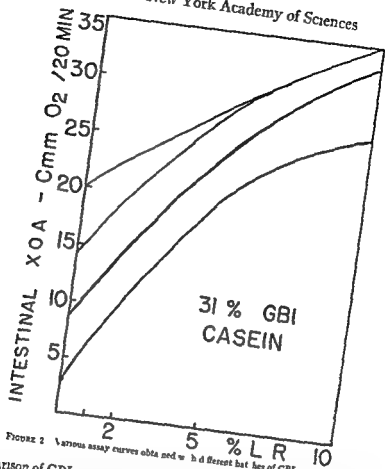


FIGURE 2 Various assay curves obtained with different batches of GBI casein

A comparison of GBI casein with an acid washed casein Labco (Borden Co) showed the latter to be far superior. Control intestinal XO values were 14 and 2 respectively for the two caseins. A control diet containing 24 per cent Labco casein has consistently given intestinal XO values of less than 2 and the bioassay curve obtained with this casein is the lower curve shown in the presence of the tenfold change indicated.

Diets with 24 and 31 per cent casein yielded the lower curve. The 24 per cent casein diet with additional protein gave a similar assay as the 31 per cent Labco casein diet.

Xanthine oxidase is apt to be localized in the mucosa of the intestine since muscles are generally free of this enzyme. To avoid possible removal of exposed mucosa by blotting the sheet and washed intestine the contents were extracted more adopted for routine testing. The 24 per cent casein diet was more erratic.

TABLE 3
ISOLATION PROCEDURE FOR XO

(1)	c	a	a	x	10	x	100	25
(2)								
(3)								
(4)								
(5)								
(6)								
(7)								

and (4) some dark green leafy vegetables. Foods which were essentially inactive included (1) muscle meats and fish, and (2) fruits and berries. The substance present in liver residue and foods, which was responsible for this response could not be identified with any factor known to be required in animal nutrition. Nor could it be identified with the antithyrototoxic factor present in liver residue.²⁴

The material present in soy flour giving positive results in the isolation procedures in which the purification by bioassay. The major steps of the isolation procedure are shown in TABLE 3. Two active barium salts were separated from the final barium precipitate. One was in

crystallized from hot water, lost a significant proportion of its weight on ashing, and contained about 17 per cent molybdenum. Although the purification procedure was not designed to yield inorganic material specifically, nevertheless the isolated substances were molybdate or molybdate complex salts.

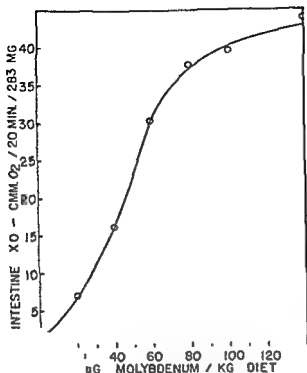
The identification of these substances as molybdates was aided by the concurrent studies of De Renzo *et al.*²⁵ Using the same assay procedure they found that the biological activity was retained in a liver residue ash, and they identified molybdenum as the active component of the ash by testing each of its constituents.

The biological activity of sodium molybdate is shown in FIGURE 4. Saturation levels of intestinal xanthine oxidase (above 30) were obtained with diets containing less than 0.1 mgm molybdenum per kgm of diet. Higher concentrations of dietary molybdenum gave intestinal XO values averaging between

lower level

— part of the active material
all of the biological
molybdenum content.
was the case

(1) The activity of the isolated material or of a large number of impure frac



of sodium molybdate showing the response of intestinal xanthine oxidase to in the diet

re sodium molybdate when tested on the basis
 of the biological activity of the isolated ma-

terence no essential or synergistic organic
 molybdenum during the isolation pro-

lated material by extracting it as
 moved the biological activity with

isolated material as an insoluble
 the biological activity
 than enough molybdenum

xanthine oxidase factor is the trace
 to be required by some plants
 in of a role for molybdenum
 um with an enzyme

TABLE 3

ISOLATION PROCEDURE FOR XOF

-
- | | | |
|---------------|--|---------------------|
| (1) Soy flour | reflux with 10 vol of 1N HCl and filter | |
| (2) Filtrate | adsorb activity on charcoal and eluate with ammonia | |
| (3) Eluate | acidify, adsorb on alumina column and elute fractionally with 1N ammonia | |
| (4) T | | Wash with 0.2 N HCl |
| (5) | | |
| (6) | | |
| (7) | | |
-

and (4) some dark green, leafy vegetables. Foods which were essentially inactive included (1) muscle meats and fish, and (2) fruits and berries. The substance present in liver residue and foods, which was responsible for this response could not be identified with any factor known to be required in animal nutrition. Nor could it be identified with the antithyrototoxic factor present in liver residue.²⁴

Isolation and identification of YOF. The material present in soy flour giving this response was isolated by a series of fractionation procedures in which the active material was followed throughout its purification by bioassay. The major steps of the isolation procedure are shown in TABLE 3. Two active barium salts were separated from the final barium precipitate. One was insoluble in water, lost little weight on ashing, and contained 45 per cent barium and 24 per cent molybdenum. Spectrographically, it contained only Ba and Mo in amounts greater than 0.1 per cent. The other barium salt could be

the isolated substances were molybdate or molybdate complex salts.

The identification of these substances as molybdates was aided by the concurrent studies of De Renzo *et al.*²⁵ Using the same assay procedure, they found that the biological activity was retained in a liver residue ash, and they identified molybdenum as the active component of the ash by testing each of its constituents.

The biological activity of sodium molybdate is shown in FIGURE 4. Saturation levels of intestinal xanthine oxidase (above 30) were obtained with diets containing less than 0.1 mgm molybdenum per kgm of diet. Higher concentrations of dietary molybdenum gave intestinal XO values averaging between 30 and 45, but they were not consistently proportional to the molybdenum content of the diet. Hence values above 30 were considered to be saturation levels of intestinal XO, and assays falling within this range were repeated at a lower level.

It was established molybdenum as a major component of the active material.

- (1) The activity of the isolated material or of a large amount

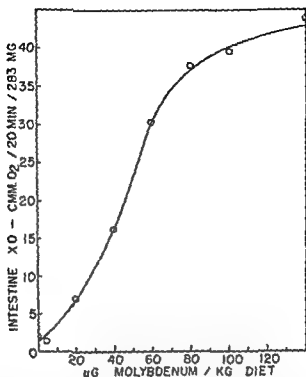


FIGURE 4. Assay curve with and without molybdate showing the response of intestinal xanthine oxidase to increasing amounts of molybdenum in the diet.

tions was identical with that of pure sodium molybdate when tested on the basis of their respective molybdenum contents.

(2) The molybdenum content and the biological activity of the isolated material were not decreased by ashing. Hence no essential or synergistic organic component was carried along with the molybdenum during the isolation procedure.

(3) A removal of molybdenum from the isolated material by extracting it as an ether-soluble thiocyanate complex also removed the biological activity with the molybdenum.

(4) Precipitation of molybdenum from the isolated material as an insoluble complex with α -benzoinoxime also precipitated the biological activity.

(5) Liver residue and soy flour contained more than enough molybdenum to account for their biological activities.

These results leave no doubt that the xanthine oxidase factor is the trace element molybdenum. Molybdenum is known to be required by some plants and microorganisms, but this is the first demonstration of a role for molybdenum in animal nutrition and is the first association of molybdenum with an enzyme.

It appears to be a part of the enzyme molecule, since purified preparations of xanthine oxidase contain molybdenum. This was demonstrated previously¹⁵ by the assay procedure, and has been confirmed more recently by chemical determinations.

Studies of molybdenum deficiency in plants²⁶⁻²⁸ and *Aspergillus niger*²⁹ have demonstrated a requirement for molybdenum in the reduction of nitrate. Xanthine oxidase³⁰ and aldehyde oxidase³¹ are capable of utilizing nitrate or organic nitro compounds³²⁻³⁴ as electron acceptors during the oxidation of purine or aldehyde substrates. Nitrate is thereby reduced to nitrite,³⁰ and organic nitro compounds are reduced to hydroxylamino derivatives.³² It seems probable, therefore, that the reduction of nitrates by plants, molds, etc., is effected by a molybdenum containing enzyme similar to but not necessarily identical with xanthine oxidase.

References

- 1 WESTERFELD W W, J M MCKIBBIN, J C ROEMMELT & M F HILFINGER 1949 *Am J Physiol* 167 184
- 2 AXELROD A E & C A ELVENJEM 1941 *J Biol Chem* 140 725
- 3 MCKIBBIN J M, F R E & T J W 1945 *Science* 101 493
- 4 WESTERFELD W W 1950 *Soc Exptl Biol Med* 71 181
- 5 WESTERFELD W W 1950 *Proc Soc Biol Chem* 184 203
- 6 WESTERFELD W W 1951 *Biol Chem* 184 163
- 7 WESTERFELD W W & D A RICHERT 1951 *Science* 109 68
- 8 VANDERLINDE R E & W W WESTERFELD 1950 *Endocrinology* 47 265
- 9 MEIKLEHAM V I C, WELLS D A, RICHERT & W W WESTERFELD 1951 *J Biol Chem* 192 651
- 10 EDWARDS S & W W WESTERFELD 1952 *Proc Soc Exptl Biol Med* 79 57
- 11 DOISY R J & W W WESTERFELD 1952 *Proc Soc Exptl Biol Med* 80 203
- 12 RICHERT D A & W W WESTERFELD 1952 *J Biol Chem* 199 879
- 13 WESTERFELD W W, D A RICHERT & M F HILFINGER 1950 *Cancer Research* 10 486
- 14 WESTERFELD W W & D A RICHERT 1951 *J Biol Chem* 192 35
- 15 RICHERT D A & W W WESTERFELD 1951 *J Biol Chem* 192 49
- 16 RICHERT D A & EDWARDS & W W WESTERFELD 1949 *J Biol Chem* 181 255
- 17 WESTERFELD W W & D A RICHERT 1952 *J Biol Chem* 199 393
- 18 REMY C, D A RICHERT, W W WESTERFELD & J TRITPERMAN 1950 *Proc Soc Exptl Biol Med* 73 573
- 19 RICHERT D A, R VANDERLINDE & W W WESTERFELD 1950 *J Biol Chem* 188 261
- 20 RICHERT D A & W W WESTERFELD 1951 *Proc Soc Exptl Biol Med* 76 252
- 21 REMY C, D A RICHERT & W W WESTERFELD 1951 *J Biol Chem* 193 649
- 22 REMY C & W W WESTERFELD 1951 *J Biol Chem* 193 659
- 23 WESTERFELD W W & D A RICHERT 1952 *J Biol Chem* 199 819
- 24 DE RENZO E, C E KALEITA, P G HEYTLER, J J OLESON, H L HUTCHINGS & J H WILLIAMS 1953 *J Am Chem Soc* 75 753
- 25 MULDER E G 1948 *Plant and Soil* 1 94
- 26 EVANS H J, E R PURVIS & F E BEAR 1951 *Plant Physiol* 46 555
- 27 HEWITT E J & E W JONES 1947 *J Pomol Hort Sci* 23 254
- 28 NICHOLAS D J D 1950 *J Sci Food Agr* 1 339
- 29 DIXON M & S THURLOW 1924 *Biochem J* 18 989
- 30 BERNHEIM F 1928 *Biochem J* 22 344
- 31 DIXON M 1926 *Biochem J* 20 703
- 32 BUEDELING E & N JOLLIFFE 1946 *J Pharmacol* 88 300

STUDIES ON THE NATURE OF THE XANTHINE OXIDASE FACTOR

By Edward C. De Renzo

Leder's Laboratories Division, American Cynamid Company, Pearl River, N. Y.

I should like to extend my thanks to Doctor Schwarz and to Doctor Westerfeld for the opportunity of presenting a brief summary of some of our findings on the nature of the xanthine oxidase factor. I should like to extend special thanks to Doctor Westerfeld for his gracious acknowledgment of our contribution.

When we first became interested in this dietary factor, a study of the literature revealed that the Syracuse workers were practically alone in thinking about a substance other than adequate protein or riboflavin which was concerned with maintaining tissue xanthine oxidase values. In order to satisfy ourselves that such a factor actually existed, we attempted to reproduce the experiments of

response in the intestinal xanthine oxidase value to varying amounts of dietary liver residue and, in addition, rats receiving the control diet alone showed relatively high levels of this enzyme. After a brief talk with Doctors Westerfeld and Richert, our problems with the assay were satisfactorily unraveled.

TABLE 1 shows the basal diet¹ used in these studies. As reported by the Syracuse workers, if weanling rats are given this diet for two weeks, analysis of the small intestine for xanthine oxidase by the manometric technique² will yield a value of about 5 cmm. oxygen uptake/20 min./250 mgm. intestine. Supplementation of this diet with 10 per cent liver residue produces a five- to sevenfold increase in the xanthine oxidase value. TABLE 2 shows the response of rat intestinal xanthine oxidase to varying amounts of liver residue in the diet thus confirming the work already reported.¹ It should be mentioned however that we sometimes experienced difficulty in obtaining xanthine oxidase values proportional to the lower amounts (i.e., 2.5 per cent) of a liver residue supplement. Nevertheless there was never any doubt about the enhancement obtained with a high level (i.e., 10 per cent) of liver residue and isolation studies were therefore undertaken.

We chose liver residue as our source material since the factor was present in abundant amounts in this material. Early in the work it was found that active extracts of xanthine oxidase factor could be obtained by simply autolyzing liver residue with water. The factor in the extract as well as in the crude liver residue was remarkably stable to acid or alkaline treatment and when the activity was not destroyed by boiling with concentrated sulfuric acid our suspicions as to the nature of the factor were aroused. We therefore assayed the active extracts and liver residue, and when the ashed materials were tested no diminution of activity was encountered. These data are shown in

TABLE 3

¹The special assistance of Mr. E. Kabala and Mr. P. L. Hoyer in the performance of these experiments gratefully acknowledged.

TABLE 1
BASAL DIET USED IN STUDIES ON XOF

Cerelose	65
Purified casein (Labco)	24
Crisco	4
Cod liver oil	1
Wesson oil	2
Salts (Phillips and Hart)	4
Vitamins/kgm diet	
Riboflavin, 4 mgm	
Thiamin HCl, 4 mgm	
Pyridoxine HCl, 4 mgm	
Ca Pantothenate, 10 mgm	
Nicotinamide, 25 mgm	
Choline chloride, 1000 mgm	

TABLE 2
RESPONSE OF RAT INTESTINAL XANTHINE OXIDASE VALUES TO LIVER RESIDUE IN THE DIET

Amount of liver residue added to basal diet (per cent)	Av X O value c mm O ₂ uptake/20 min /250 mgm intestine
None	5 0
2	10 2
5	22 0
10	29 8

TABLE 3
EFFECT OF LIVER RESIDUE FRACTIONS ON RAT INTESTINAL XANTHINE OXIDASE VALUES

Supplement added to basal diet	Av X O value c mm O ₂ uptake/20 min /250 mgm intestine
None	4 4
10% liver residue (LR)	25 6
Liver residue water extract (LRE)	32
Ash of LR	28 2
Ash of LRE	26 9

A spectrographic analysis (TABLE 4) of the ash of the active extract revealed the presence of many elements. The activity of the ash was then found to be replaceable by Hoagland's A-Z solution (50 ml/kgm diet), an inorganic solution containing trace elements and used in studies of plant nutrition.⁴ Single salts were then fed in varying amounts in an attempt to ascertain the active substance. On the basis of the elemental composition of the ash and Hoagland's solution and the salt mixture of the basal diet, our studies were restricted to relatively few salts. To our surprise, molybdenum salts were found to be responsible for this effect. These data are shown in TABLE 5. No other salts studied exhibited this effect, and the activity of the ash of active extracts or liver residue could be totally accounted for on the basis of its molybdenum content. Furthermore, and also to our surprise, when an ash of the basal diet was assayed spectrographically, molybdenum was not detected (TABLE 6).

We have attempted to study the effects of molybdenum on milk as well as

TABLE 4
SPECTROGRAPHIC ANALYSIS OF LIVER RESIDUE ASH

Element detected	Approximate per cent (unless stated as parts per million ppm)
Al	0.3
Sb	Trace
Ba	0.1
B	1
Ca	1
Cr	30 ppm.
Co	0.1 ppm.
Cu	0.1
Fe	1
Pb	0.3
Mg	10
Mn	30 ppm.
Mo	0.3
Ni	30 ppm.
P	3
K	3
Si	10
Ag	30 ppm.
Na	30
Sn	0.03
Tl	10 ppm.
V	0.03
Zn	0.1

TABLE 5
EFFECT OF CERTAIN SALTS ON RAT INTESTINAL VANTHINE OXIDASE VALUES

Supplement added, 3 gm. diet	% V.O. value 1 mm. Or up- take 25 min. / 250 mgm. intestine
None	7.1
50 mL. Hengland's solution	21.4
AlCl ₃ 6H ₂ O 200 mgm.	5.3
CoCl ₂ 6H ₂ O 200 mgm.	1.1
Cr(NO ₃) ₃ 9H ₂ O 200 mgm.	3.7
SnCl ₄ 5H ₂ O 200 mgm.	3.4
SeCl ₄ 6H ₂ O 200 mgm.	3.7
H ₃ PO ₄ 100 mgm.	8.6
NaBr 25 mgm.	4.9
K ₂ Cr ₂ O ₇ 25 mgm.	4.8
Ti(OH) ₄ 25 mgm.	6.7
Ni(NO ₃) ₂ 6H ₂ O 10 to 100 mgm.	9.5
BaCl ₂ 10 to 50 mgm.	3.7
NaAlO ₂ 2H ₂ O 0.05 to 100 mgm.	27.3

TABLE 6
ELEMENTS DETECTED IN THE BASAL DIET ASH SHOWING THE ABSENCE OF POLYBROMIN

Al	Na
Ba	Fe
Ca	Mg
Cr	Si
Cu	Ag
Fe	As
Pb	Co
Mg	Zn
Si	

intestinal xanthine oxidase *in vitro* Varying concentrations of molybdate were added to dialyzed or undialyzed active enzyme preparations and enzyme preparations from rats receiving the basal diet (*i.e.*, deficient in molybdenum) No enhancement of activity has yet been observed under these conditions with any of these enzyme preparations

In preliminary experiments, we have found that the feeding of sodium tung

U
nal xanthine oxidase preparations has been observed *in vitro*

We have also demonstrated an *in vivo* stimulatory effect of molybdenum on mouse intestinal xanthine oxidase and therefore feel that the participation of Mo in animal nutrition and metabolism has been firmly established Further attempts are now in progress to demonstrate the precise role of molybdenum on the activity of xanthine oxidase

References

- 1 RICHERT, D A & W W WESTERFELD 1951 J Biol Chem 192 49
- 2 AXELROD, A E & C A ELVENJEM 1941 J Biol Chem 140 725
- 3 HOAGLAND, D R & W C SNYDER 1933 Proc Am Soc Hort Sci 30 288
- 4 PHILLIPS, P H & E B HART 1935 J Biol Chem 109 657

THE EFFECTS OF LIVER DISEASE ON CERTAIN ASPECTS OF TOCOPHEROL METABOLISM IN MAN*

By Gerald Klatskin

Yale University School of Medicine, New Haven, Conn

The purpose of the present investigation was to characterize the effects of liver disease on certain aspects of tocopherol metabolism in man. Our interest in this problem was stimulated by the observation that chronic biliary fistula leads to vitamin E deficiency in experimental animals,^{1, 2} an effect thought to be the consequence of impaired tocopherol absorption. Although clinical evidence of vitamin E deficiency has not been recognized in man, a similar defect in absorption has been postulated to account for the low levels of plasma tocopherol found in liver disease.^{3, 4} The subnormal rise in the plasma level following the administration of tocopherol in such cases^{3, 4} would appear to lend support to this hypothesis. It is obvious, however, that the plasma level reflects not only the rate of tocopherol absorption in the intestine, but also its rate of storage and utilization in the tissues. Unfortunately, little is known about these aspects of tocopherol metabolism in man, so that the significance of low plasma levels has remained obscure. However, recent evidence implicating tocopherol deficiency in the pathogenesis of experimental dietary hepatic necrosis^{5, 6} has served to emphasize the possible importance of this problem. The following studies were undertaken, therefore, with the hope of shedding further light on the interrelationships between hepatic dysfunction and the absorption, utilization, and storage of tocopherol in liver disease.

Results

Plasma tocopherol. Analyses of plasma confirmed previous reports⁴ that the average tocopherol level in liver disease is significantly lower than in normal healthy adults (FIGURE 1). It was found, however, that the individual values varied widely often falling within the normal range, and that the average did not differ significantly from that of a group of randomly selected hospitalized convalescent controls. Moreover, there was no correlation between the concentration of tocopherol and the depth of jaundice or the degree of hepatic dysfunction as judged from the serum levels of bilirubin, cholesterol, and albumin (FIGURE 2).

These observations suggested that the decrease in plasma tocopherol in liver disease was related to some extrahepatic factor such as impaired absorption rather than to a metabolic defect in the liver. However a depletion of the tissues and plasma due to a dietary deficiency of tocopherol had to be excluded, since many of the subjects in both the liver disease and convalescent control groups had subsisted on grossly deficient diets for long periods.

Saturation tests. If the fall in plasma tocopherol were indeed related to such a deficiency repletion of the tissues might be expected to effect a sustained rise in the plasma tocopherol level. For that reason, the effects of large amounts

* This investigation was supported by a research grant A-4 (C7) from the National Institute of Arthritis and Metabolic Diseases of the National Institutes of Health, U. S. Public Health Service.

intestinal xanthine oxidase *in vitro*. Varying concentrations of molybdate were added to dialyzed or undialyzed active enzyme preparations and enzyme preparations from rats receiving the basal diet (*i.e.*, deficient in molybdenum). No enhancement of activity has yet been observed under these conditions with any of these enzyme preparations.

In preliminary experiments, we have found that the feeding of sodium tungstate to rats receiving minimal amounts of molybdenum inhibits the stimulation of intestinal xanthine oxidase. This appears to be an interesting sort of ion antagonism. However, no inhibitory effect of tungstate on milk or intestinal xanthine oxidase preparations has been observed *in vitro*.

We have also demonstrated an *in vivo* stimulatory effect of molybdenum on mouse intestinal xanthine oxidase and therefore feel that the participation of Mo in animal nutrition and metabolism has been firmly established. Further attempts are now in progress to demonstrate the precise role of molybdenum on the activity of xanthine oxidase.

References

- 1 RICHERT, D. A. & W. W. WESTERFELD. 1931. J. Biol. Chem. 192: 49.
- 2 AXELROD, A. E. & C. A. ELVEHJEM. 1941. J. Biol. Chem. 140: 723.
- 3 HOAGLAND, D. R. & W. C. SNYDER. 1933. Proc. Am. Soc. Hort. Sci. 30: 288.
- 4 PHILLIPS, P. H. & E. B. HART. 1935. J. Biol. Chem. 109: 657.

THE EFFECTS OF LIVER DISEASE ON CERTAIN ASPECTS OF TOCOPHEROL METABOLISM IN MAN*

By Gerald Klatskin

Yale University School of Medicine, New Haven, Conn

The purpose of the present investigation was to characterize the effects of liver disease on certain aspects of tocopherol metabolism in man. Our interest in this problem was stimulated by the observation that chronic biliary fistula leads to vitamin E deficiency in experimental animals,^{1,2} an effect thought to be the consequence of impaired tocopherol absorption. Although clinical evidence of vitamin E deficiency has not been recognized in man, a similar defect in absorption has been postulated to account for the low levels of plasma tocopherol found in liver disease.^{3,4} The subnormal rise in the plasma level following the administration of tocopherol in such cases^{5,6} would appear to lend support to this hypothesis. It is obvious, however, that the plasma level affects not only the rate of tocopherol absorption in the intestine, but also its rate of storage and utilization in the tissues. Unfortunately, little is known about these aspects of tocopherol metabolism in man, so that the significance of low plasma levels has remained obscure. However, recent evidence implicates tocopherol deficiency in the pathogenesis of experimental dietary hepaticrosis^{7,8} has served to emphasize the possible importance of this problem. Further studies were undertaken, therefore, with the hope of shedding further light on the interrelationships between hepatic dysfunction and the absorption, utilization, and storage of tocopherol in liver disease.

Results

Plasma tocopherol. Analyses of plasma confirmed previous reports⁴ that the average tocopherol level in liver disease is significantly lower than in normal healthy adults (FIGURE 1). It was found, however, that the individual values varied widely, often falling within the normal range, and that the average did not differ significantly from that of a group of randomly selected hospitalized convalescent controls. Moreover, there was no correlation between the concentration of tocopherol and the depth of jaundice or the degree of hepatic dysfunction as judged from the serum levels of bilirubin, cholesterol, and albumin (FIGURE 2).

These observations suggested that the decrease in plasma tocopherol in liver disease was related to some extrahepatic factor such as impaired absorption, rather than to a metabolic defect in the liver. However, a depletion of the tissues and plasma due to a dietary deficiency of tocopherol had to be excluded, since many of the subjects in both the liver disease and convalescent control groups had subsisted on grossly deficient diets for long periods.

Saturation tests. If the fall in plasma tocopherol were indeed related to such a deficiency, repletion of the tissues might be expected to effect a sustained rise in the plasma tocopherol level. For that reason, the effects of large amounts

* This investigation was supported by a research grant (A-4147) from the National Institute of Health and the National Institutes of Health U. S. Public Health Service.

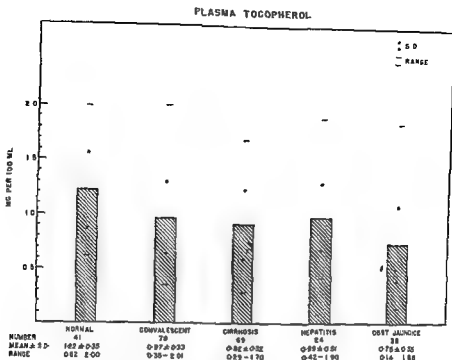


FIGURE 3

of orally administered tocopherol were investigated. (The 4 gm. dose of acetate employed approximated the estimated total body content of tocopherol).¹²

It can be seen in A and B of FIGURE 3 that, with the exception of the obstructive jaundice group, the subjects with liver disease showed as great a rise in plasma concentration during tocopherol administration as the normals, suggesting that absorption from the intestine was not impaired. However, as is evident in D of FIGURE 3, the rise was sustained in the cirrhotic group only. In the latter, moreover, the absolute increase in concentration over the control level actually exceeded that in the normals (C of FIGURE 3).

These data suggested that dietary deficiency was at least one of the factors responsible for the low plasma tocopherol levels in cirrhosis. If it played any role in the other types of liver disease investigated, it was obscured by other more important factors.

Tolerance tests To further evaluate the factor of intestinal absorption, tolerance tests based on plasma curves following the oral administration of tocopherol were carried out. These confirmed previous reports⁴ that the curves in liver disease tended to be lower and flatter than normal (A of FIGURE 4). Further analysis, however, revealed that while the peak values attained were low (B of FIGURE 4), the increases above the initial fasting level (C of FIGURE 4), and the mean rises in concentration estimated from the areas under the curves (D of FIGURE 4), were within normal limits, except in the case of obstructive jaundice.

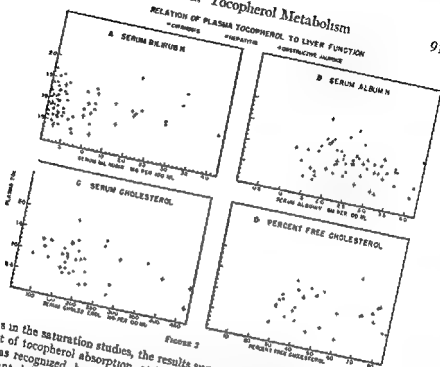


FIGURE 3

As in the saturation studies, the results suggested that there was no impairment of tocopherol absorption, at least in the cirrhotic and hepatitis groups. It was recognized, however, that estimates based on plasma curves did not warrant definite conclusions regarding intestinal absorption, unless paralleled by measurements of tocopherol excretion. For that reason, it was necessary to carry out a number of balance experiments. Since preliminary investigation revealed that little or no tocopherol was excreted in the urine, these were based on analyses of feces.

Fecal tocopherol. To our surprise, it was found that subjects with liver disease regularly excreted a smaller fraction of ingested tocopherol than normals, both on their usual diets (A and B of FIGURE 5) and following the ingestion of large tocopheryl acetate supplement (C of FIGURE 5). Several possible explanations for the low fecal tocopherols in liver disease were considered.

- (1) A larger fraction of ingested tocopherol was absorbed, (2) A fraction of the ingested tocopherol was excreted as the acetate and, therefore, escaped detection in the Emmene Engel reaction, which served as the basis for the analytical procedure employed,¹⁰
- (3) There was interference with some as yet unrecognized normal mechanism for the re-excretion of tocopherol occurred in the intestinal tract of the normals
- (4) Synthesis of tocopherol occurred in the subjects with liver disease, and was diminished in the subjects with liver disease,

EFFECT OF "SATURATING" DOSES OF TOCOPHEROL
4 GM OF TOCOPHERYL ACETATE OVER AN 8 DAY PERIOD

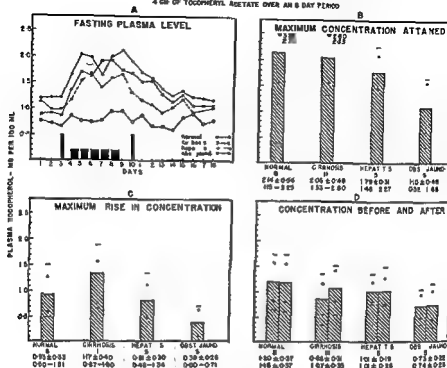


FIGURE 3

(5) There was an increased rate of tocopherol breakdown in the intestinal tract

(1) *Increased absorption* If the subjects with liver disease did in fact absorb a larger fraction of ingested tocopherol, it would have to be assumed that the relatively low or normal plasma curves observed during tocopherol absorption (FIGURE 4) were the consequence either of an increased rate of utilization or of a more rapid diversion of tocopherol to the tissues. Under these conditions, loading of the plasma and tissues with large amounts of tocopherol might be expected to result in a relative decrease in the rate of utilization and/or transfer

curves can be used as an index, did it alter the rate of utilization. Unfortunately, more direct means for measuring utilization could not be devised. Nevertheless, it was concluded tentatively that the low fecal tocopherol levels in liver disease were not due to an increase in tocopherol absorption

TOCOPHEROL TOLERANCE TESTS
800 MG TOCOPHERYL ACETATE DAILY

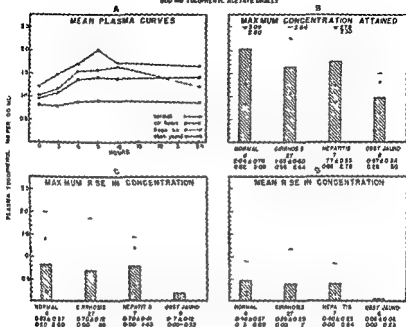


FIGURE 4

(2) *Tocopheryl esters* When the feces were saponified to free tocopherol

organisms (B of FIGURE 7) indicating that the esterases responsible for this

FECAL EXCRETION OF TOCOPHEROL

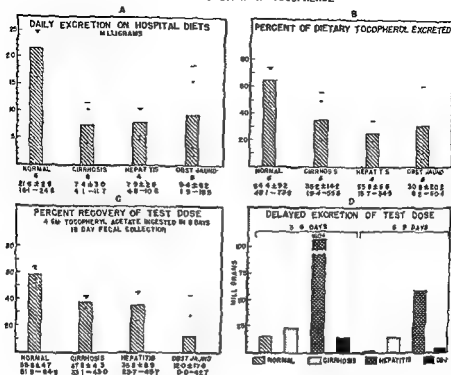


FIGURE 5

TOCOPHEROL TOLERANCE TESTS BEFORE AND AFTER "SATURATION"

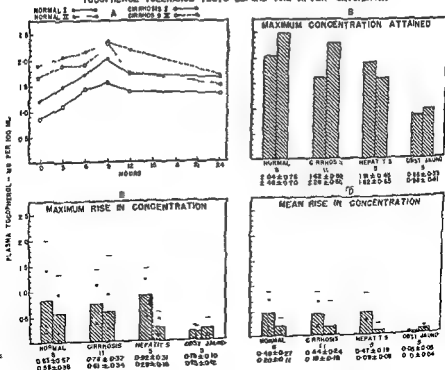
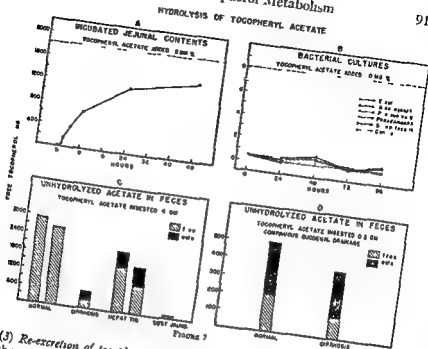


FIGURE 6



(3) *Re-excretion of tocopherol* The possibility that there was interference with some normal mechanism for the re-excretion of tocopherol into the intestinal tract, which led to a reduction in fecal tocopherol, was suggested by the observation that the amount excreted in liver disease remained significantly above the baseline level for at least 9 days following an oral test dose of tocopherol (D of FIGURE 5). Partial confirmation of such a mechanism was obtained from analyses of T tube bile in subjects with obstructive jaundice. These revealed concentrations of tocopherol comparable to those in plasma (A of FIGURE 8). However, it was not possible to demonstrate any interference with this enterohepatic circulation of tocopherol in liver disease. Thus, analyses of duodenal contents aspirated for a period of 24 hours following instillation of tocopherol distal to an occlusive balloon in the jejunum failed to reveal any difference in the amount recovered in normal and cirrhotic subjects (C of FIGURE 8). Moreover, the fact that the amount recovered was small and the observation that the concentration in T tube bile did not increase following the ingestion of tocopherol (B in FIGURE 8) suggested that the bile contributed very little to the total fecal tocopherol in the previously described balance experiments.

It was concluded, therefore that interference with the enterohepatic circulation of tocopherol was not responsible for the low fecal levels found in liver disease. The possibility of a defect in its re-excretion, however, through the intestinal mucosa could not be excluded, although it appeared unlikely.

BILIARY EXCRETION OF TOCOPHEROL

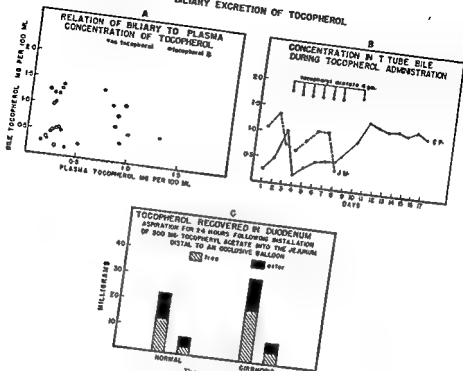


FIGURE 8

STABILITY OF TOCOPHEROL DURING INCUBATION AT 37°C

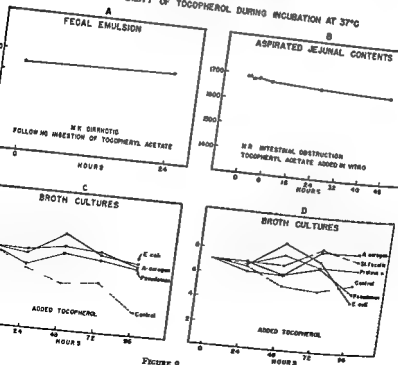


FIGURE 9

(4) *Tocopherol synthesis* The possibility of tocopherol synthesis in the intestinal tract was investigated, although there was no previous evidence to indicate that it was a factor in determining the level of tocopherol in the feces. Attempts to demonstrate such synthesis in cultures of a broad spectrum of aerobic and anaerobic enteric organisms grown in a variety of enriched media, or in incubated emulsions of feces and aspirated intestinal contents, were uniformly unsuccessful.

(5) *Tocopherol "destruction"* Largely by exclusion, an increased rate of destruction in the intestinal tract appeared to be the most likely explanation for the low fecal tocopherol in liver disease. It was not possible, however, to demonstrate this by direct experiment. Thus, there was no loss of tocopherol during its prolonged incubation in fecal emulsions (A of FIGURE 9), aspirated jejunal contents (B of FIGURE 9), or cultures of enteric organisms (C and D of FIGURE 9). Nevertheless, it must be emphasized that these experimental conclusions by no means duplicated those in the intestinal tract so that differences in tocopherol destruction were not excluded. The recent observations of Rosenkrantz and his associates¹ are highly significant in this connection. These investigators found that an appreciable fraction of the tocopherol in normal feces was excreted as the quinone a form not detectable by the Emmerie Engel reaction. It is conceivable, therefore, that a more rapid oxidation of tocopherol to tocopherylquinone in the intestinal tract was responsible for the low fecal values obtained by the Emmerie-Engel reaction in the present experiments. Further studies along these lines are contemplated.

Summary

In summary, then, previous reports of low plasma tocopherol levels in liver disease have been confirmed. The evidence presented, however, suggests that these low levels are not the result of impaired absorption but are, rather, the consequence of an increased rate of tocopherol breakdown in the intestinal tract. Moreover in some instances dietary deficiency appears to be a contributory factor.

Acknowledgment

The author is indebted to Doctor David W. Molander, Doctor Willard A. Krehl, Doctor Daniel Marshall and Miss Rita Pacelli for their assistance in carrying out these studies.

References

- BRINKHADS K M & E D WARNER 1941 Muscular dystrophy in biliary fistula dogs possible relationship to vitamin E deficiency. *Am J Path* 17: 81
- GRAVES J D & C L A SCHMIDT 1937 Relation of bile to absorption of vitamin E in the rat. *Proc Soc Exptl Biol Med* 37: 40
- KLATSJIN G & W A KREHL 1951 The significance of the plasma tocopherol concentration and of tocopherol tolerance tests in liver disease. *J Clin Invest* 30: 1523
- POPPER H A, DUBIN F, STEINMANN & F P HESSER 1949 Plasma tocopherol levels in various pathologic conditions. *J Lab Clin Med* 34: 648
- GEORGY P & H GOLDBLATT 1949 Further observations on the production and prevention of dietary hepatic injury in rats. *J Exptl Med* 89: 245
- HINISWORTH H P & O LYNDAN 1949 Dietetic necrosis of the liver: the influence of alpha tocopherol. *Nature* 163: 30

7. SCHWARZ, K. 1949 Dietetic hepatic injuries and the mode of action of tocopherol
Ann N Y Acad Sci 51: 225
8. QUACKER, H. 1950 Die Bedeutung des Vitamin E für die Ernährung des Menschen
Dtsch. Ernährungsges. 1: 1
9. K. " "
10. K. " "
11. REICHERT, L. 1950 Die Bedeutung des Vitamin E für die Ernährung des Menschen
Dtsch. Ernährungsges. 1: 1
- tion in identification of tocopherol compounds in feces J Biol Chem 192: 9

PHOSPHOLIPIDE SYNTHESIS IN LIVER METABOLISM*

By W. E. Cornatzer
University of North Dakota Medical School Grand Forks N. D.

Phospholipide synthesis is a primary function of all cells in the body. They make up part of the cell structure, being found in both the cytoplasm and nucleus. Mitochondria and large granules are very rich in phospholipides. These intracellular structures contain enzymes which can oxidize fatty acids and other metabolites. The liver is the main source of phospholipides in plasma. Plasma phospholipides show a direct reflection of turnover in the liver. The choline-containing phospholipides are probably an integral part of enzyme systems necessary for the oxidation of fats and fatty acids. The lipotropic effect of lecithin appears to act on the metabolism of fatty acids in the liver, rather than by enhancing their mobilization in the form of plasma phospholipides. The composition of liver lipides can be altered by the dietary intake of proteins. There is usually a decrease in the level of total phospholipides in the liver of animals maintained on a low protein diet. The decrease is especially marked in the lecithin fraction with a consequent lower ratio of the choline-containing to the total phospholipides. Various studies comparing the rate of incorporation of P^{32} into the liver lipides of animals on various experimental diets have been made.

The phospholipide turnover in the plasma of normal individuals varies widely but is rather constant in the same individual over a period of many months. The effect of a low protein diet (7.6 per cent) or a normal diet for 9 days seems to have very little change in the rate of synthesis of the plasma phospholipide in man. The phospholipides of the plasma are primarily choline-containing, but in the liver, both choline and noncholine-containing are abundant. Attempts have been made to study the effects of dietary requirements on the synthesis of these two fractions in the liver. The synthesis of the choline containing fraction was practically the same in the liver of animals maintained on a low protein diet (5 per cent) as in those on a stock diet or a 25 per cent casein diet. The only instance in which a marked decrease in synthesis of the choline containing fraction was supplemented. Diethanolamine apparently acts as a metabolic antagonist of ethanolamine for the formation of lecithin as well as the natural cephalin. The rate of lecithin synthesis by the liver of the rat is apparently related to the activity of choline oxidase, or to the role of natural inhibitors such as fatty acids. This diminished choline oxidase activity permits an adequate rate of synthesis of phospholipides containing choline. It is impossible to produce a "fatty" liver by dietary means in the liver of the guinea pig, since this enzyme is not present in the liver cell is more efficient with the lecithin molecule when the fatty even though a decrease in the total phospholipids.

* This is a preliminary report of the author's work supported by a Fellowship of the Society.

TABLE 1
PHOSPHOLIPIDE SYNTHESIS IN THE RAT AND GUINEA PIG ON STOCK DIET*

Animal	No	Body weight gm	Phospholipid P			Phospholipide-P/Nitrogen	Relative radioactivity per gm of fat free tissue
			mgm	Relative specific activity			
				overall	per gm of fat free tissue		
Rat	8	278	17.4	11386	11040	0.054	0.68
Guinea pig	6	989	33.5	11258	0.009	11042	0.30

* Animals killed 8 hours after the injection of P^{32}

A comparison of phospholipide synthesis in the guinea pig and rat has been made in an attempt to determine the role of choline oxidase in lipid phosphorylation (TABLE 1)¹⁸ In rats and guinea pigs maintained on stock diets the phospholipide P/N was unaltered. The relative specific activities of the phospholipides of the guinea pig are only slightly decreased from those of the rat. This difference becomes less when the relative radioactivity values of the guinea pig liver lipides are calculated for 1 gm. of fat free tissue, which should represent the activity of a definite weight of tissue in synthesizing phospholipides. We may conclude that the synthesis of phospholipides by the

heavy metals, has long been known. Infections in different parts of the body, as well as parasitization of the liver cells themselves, alter liver functions.¹⁹ The phospholipide synthesis in the plasma of untreated cirrhotic patients is very similar to that found in normal persons.²⁰ The phospholipide turnover in the liver of rats in experimental cirrhosis has been produced by carbon tetrachloride and has been found to be unaltered.²¹ The phospholipide

is constant when expressed as per gram of fat free tissue. Attempts to compare the rate of incorporation of P^{32} into the liver phospholipides and nucleoproteins of rats have been made (TABLE 2).²⁴ Male rats (100-110 gms.) were maintained 4-8 weeks on diets containing 25 per cent, 5 per cent Casein with respectively a low (5 per cent) or high (32 per cent) fat content. The rats injected with P^{32} and after 6 hours the inorganic P, lipides and nucleo-

activities of the phospholipide 1/mole cop...

TABLE 2

AVERAGE VALUES OF PHOSPHOLIPIDE AND "NUCLEOPROTEIN" TURNOVER IN THE LIVER OF RATS ON VARIOUS DIETS

Diet		Number of animals	Body weight gm	Total lipides gm	Phospholipide P		Nucleo-protein P		R S A Phospholipide-P/ R S A Nucleo-protein P
Protein %	Fat %				Relative specific activity		Relative specific act vit/g	Phospholipide P/ Nitrogen	
					overall	per gm of fat free tissue			
5	5	16	114	1.47	0.466	0.087	0.234	0.037	1.840
25	5	10	230	0.54	0.390	0.044	0.178	0.037	2.190
5	32	11	130	2.46	1.045	0.167	0.389	0.050	2.690

* R S A = Relative specific activity. All animals maintained on the diet 4 weeks

TABLE 3

AVERAGE VALUES FOR PHOSPHOLIPIDE AND "NUCLEOPROTEIN" TURNOVER IN THE LIVER OF RATS MAINTAINED ON A LOW PROTEIN LOW FAT DIET*

Days on diet	Number of animals	Body weight gm	Total lipides gm	Phospholipide-P		Nucleo-protein P		Phospho-lipide P/ nitrogen	R S A Phospho-lipide-P/ R S A nucleo-protein P
				Relative specific activity		Relative specific activity			
				overall	per gm. of fat free tissue				
64	11	101	2.33	0.210	0.053	0.084	0.037	2.508	
96	4	148	4.07	0.179	0.030	0.071	0.040	2.663	
114	8	126	3.21	0.200	0.032	0.093	0.039	2.175	
164	12	169	3.09	0.177	0.025	0.102	0.029	1.767	
185	6	152	2.36	0.259	0.046	0.137	0.037	2.040	
206	14	142	2.99	0.181	0.028	0.083	0.033	2.211	

* 8% protein and 5% fat

† R S A = relative specific activity

intake or by the amount of fat in the liver. Campbell and Kosterlitz²³ have shown that the phospholipide P content of a unit of liver cell is determined mainly by the dietary protein intake, a little by the fat intake, and not at all by the choline content of the diet. The ratio of phospholipide P/protein N, is constant over a wide range of dietary protein intake.²⁴

Attempts to compare the rate of phospholipide and nucleoprotein synthesis in the production of experimental cirrhosis have been made (TABLE 3). In rats maintained on a 5 per cent Casein diet for 4, 8, 13, 16, and 28 weeks, the ratios of the synthesis of phospholipides to nucleoprotein and phospholipide P/N were constant and were not related to the degree of cirrhosis or fibrosis by histological evidence. There appears to be a constant relation of the synthesis of phospholipide to that of the nucleoprotein, whether or not the liver is "fatty," fibrosed, or normal.²⁴

This constant rate of synthesis of liver phospholipide appears changed very

TABLE 4

EFFECT OF THIOUREA ON THE PHOSPHOLIPIDE TURNOVER IN THE LIVER OF RATS

Wks on exp	Substance given	No of rats	Specific activity				
			Inorganic P	Lipide P		Lipide P \times 100 inorganic P	
					% change		% change
3	None	4	152	101		67	
	Thiouracil	4	157	79	-22	50	-25
	Thiouracil*	4	164	78	-23	47	-30
5	None	4	173	115		67	
	Thiouracil	4	187	96	-16	51	-24
	Thiouracil†	4	213	110	-4	52	-22

Rats fed a high protein diet (thiouracil 10.3%) then a low protein (thiouracil 10.6%) last week of experiment

* Last day 100 mgm thiouracil by stomach tube

† Last day 100 mgm thiourea by stomach tube

TABLE 5

THE EFFECT OF THYROID GLAND ON THE PHOSPHOLIPIDE TURNOVER IN THE PLASMA OF MAN

Patient	Exp conditions	Relative specific activities of plasma phospholipids		
		24 hours	48 hours	72 hours
R B	Thyrotoxicosis After surgery	0.731	1.588	1.85
		0.292	0.805	1.03

little by dietary intake, but is influenced by metabolic requirements of the body. The thyroid gland plays a definite role in this metabolism.²⁶ In experimental animals thyroxine increases the rate of synthesis both in plasma and liver.²⁷ Thiouracil and thiourea diminish the phospholipide turnover.^{27, 28} As shown in TABLE 4, a significant decrease in the animals receiving the drugs is apparent. The plasma phospholipide turnover in a patient with clinical thyrotoxicosis showed a decreased rate of synthesis.

Lipide phosphorylation is greatest in conditions in which a single dose of lipotropic agents are administered in the presence of a "fatty" liver. The effect of a dose of choline is chiefly due to an increased rate of formation of lecithins.²⁹ The stimulating effect of various incomplete methylated ethanol amine derivatives was greatest in the choline containing fraction in rats maintained on a low protein diet.³⁰ In cirrhotic patients with fatty infiltration of the liver by biopsy a significant increase in the rate of phospholipide synthesis is usually demonstrable after a single dose of choline or methionine.³¹

The stimulating action of lipide phosphorylation in the liver has a low degree of structural specificity than other biological actions exerted by the same substance.³² Thus there are substances which, without being lipotropic, increase the turnover rate of liver phospholipides, e.g., cystine,³³ ethanolanine,^{34, 35} and diethanolanine.³⁶ In animals maintained on a low protein diet, the stimulat

TABLE 6
THE EFFECT OF CORTISONE ON THE RATIO OF PHOSPHOLIPIDE AND NUCLEOPROTEIN*
TURNOVER IN THE RABBIT*

Tissue	Number of animals	Material given	Relative specific activity of phospholipide P	Relative specific activity of nucleoprotein P
Liver	10	—	1.32	1.62
Kidney	13	Cortisone	1.62	1.94
Lung	4	—	1.94	1.05
Heart	4	Cortisone	1.05	1.00
Skeletal muscle	7	—	1.00	1.15
	4	Cortisone	0.62	0.61
	9	—	0.61	0.69
		Cortisone	0.61	0.61

* Cortisone 7.5 mgm. I.M. daily for two months

ing effect of ethanolamine was most pronounced in the noncholine-containing fraction of the liver lipides.²⁰ Diethanolamine presumably acts as a metabolic antagonist of the methyl acceptors in the synthesis of choline with the accumulation of an atypical cephalin.¹⁸ This stimulatory effect of lipid phosphorylations by lipotropic agents is somewhat related to the previous diet for it does not occur in rats on a stock diet²⁴ or in man in adequate protein intake.^{9, 10} Many other compounds have to be studied for their stimulating effect of lipid phosphorylations.²² Cortisone administered for two months in the rabbit, failed to change the rate of lipid phosphorylation in the liver (TABLE 6).¹⁷ Apparently, the rate of synthesis of phospholipide by the liver cell is rather constant when related to a unit of tissue or nucleoprotein and is not influenced by the protein content of the diet, fat content of the liver or degree of fibrosis. The stimulating effect of various lipotropic agents may represent a replacement of a deficiency of methyl donors or methyl acceptors^{12, 13} or merely a mass action effect.²⁵

References

1. SHANSON, M. A. & C. ARTOM. 1950. *J. Biol. Chem.* **187**, 281.
2. KENNEDY, E. P. & A. L. LEMNINGER. 1949. *J. Biol. Chem.* **179**, 95.
3. FISHLER, M. C. & C. ENTENMAN. M. L. MONTGOMERY & I. L. CHAIKOFF. 1943. *J. Biol. Chem.* **160**, 47.
4. ZILVERSMIT, D. B. C. FRIEDMAN & I. L. CHAIKOFF. 1948. *J. Biol. Chem.* **178**, 193.
5. CORNATZER, W. E. 1952. In *Phosphorus Metabolism*. McELROY, W. D. AND B. GLASS, Eds. 2, 243. Johns Hopkins Press, Baltimore, Md.
6. ARTOM, C. & W. H. FISHMAN. 1943. *J. Biol. Chem.* **148**, 405, 415, 423. 1947. *Ibid.* **170**, 587.
7. FISHMAN, W. H. & C. ARTOM. 1944. *J. Biol. Chem.* **154**, 109, 117. 1946. *Ibid.* **164**, 387.
8. BOLLMAN, J. L. & E. V. FLOCK. 1946. *Federation Proc.* **5**, 218.
9. CORNATZER, W. L. & D. CAYER. 1950. *J. Clin. Invest.* **29**, 534.
10. CORNATZER, W. E. D. CAYER & W. A. LAMBETH. 1951. *J. Lab. Clin. Med.* **38**, 139, 65.
11. HACK, V. H. 1947. *J. Biol. Chem.* **169**, 137. C. ARTOM. 1941. *J. Biol. Chem.* **139**, 65.
12. A. TAUBER, C. FRIEDMAN & I. L. CHAIKOFF. 1944. *J. Biol. Chem.* **156**, 385.
13. M. J. ALBRINK. 1950. *J. Clin. Invest.* **29**, 46.
14. TAUBER, A. C. FRIEDMAN, B. A. FRIES & I. L. CHAIKOFF. 1944. *J. Biol. Chem.* **156**, 165, 19.

13 ARTOM, C & W E CORNATZER 1949 Abstracts Comm First Intern Congr Biochem Cambridge, Eng 22

14 ARTOM, C & W E CORNATZER 1949 J Elisha Mitchell Sci Soc 65: 190, also unpublished data

15 ARTOM, C, W E CORNATZER, & M CROWDER 1949 J Biol Chem 180 495

16 ARTOM, C & W E CORNATZER 1952 J Biol Chem 144 401
ptl Biol Med 70 70
Unpublished data
1949 Am J Med Sci 218 500
1950 J Clin Invest 29 542
1946 Federation Proc 5 133 J L BOLLMAN & E V FLOCK 1946 J Lab Clin Med 31 478

22 CAYER, D & W E CORNATZER 1951 Gastroenterology 10 79

23 CORNATZER, W E & C ARTOM Unpublished data

24 CORNATZER, W E, J M DAVISON, & D G GALLO 1953 Federation Proc 12 191

25 CAMPBELL, R M & H M HOSTERLITZ 1952 Biochim et Biophys Acta 8 664

26 HANDLER P 1943 J Biol Chem 173 295, P HANDLER, & R H FOLLIS 1943 J Nutrition 30 669

27 FLOCK, E V, J L BOLLMAN & J BERASOV 1948 Am J Physiol 155 402

28 CORNATZER W E & C ARTOM 1949 J Elisha Mitchell Sci Soc 65 191, also unpublished data

29 ENTENMAN, C I L CHAIKOFF, & H M FRIEDLANDER 1946 J Biol Chem 162 111, H D FRIEDLANDER, I L CHAIKOFF, & C ENTENMAN 1945 J Biol Chem 158 231

30 ARTOM, C & W E CORNATZER 1948 J Biol Chem 176 949

31 WILLIAMS, J O, D CAYER & W E CORNATZER 1951 Southern Med J 44 369

32 CORNATZER W E & C ARTOM 1949 J Biol Chem 178 775

33 PERLMAN I, N STILLMAN & I L CHAIKOFF 1940 J Biol Chem 133 651

34 ARTOM, C & W E CORNATZER 1947 Abstracts Comm 17th Intern Physiol Congr Oxford, Eng 56, 1948 Federation Proc 7 143

35 PLATT, A P & R R PORTER 1947 Nature 160 905

36 ARTOM, C & W E CORNATZER 1947 J Biol Chem 171 779

37 CORNATZER W E D G GALLO J M DAVISON, & R G FISCHER 1953 Am J Med 14: 747

ANTIBIOTICS AND LIVER INJURY*

By Paul Gyorgy

Hospital of the University of Pennsylvania Philadelphia, Pa

Dietary factors determining experimental injury in rats are summarized in TABLE I

The dietary factors beneficial in the prevention of cirrhosis as the more chronic form of hepatic injury may be identified by one common denominator, *i.e.* by a sufficient supply of hypotropic factors, in particular of choline and its precursors. The benefit seen after administration of Vitamin B₁₂ may be due to its choline sparing effect. In more recent studies we found hog mucin effec

appears to improve protein utilization.

The acute form of experimental dietary hepatic injury, acute massive necrosis of the liver, may be prevented by the sulfur containing amino acids, cystine or methionine, or by vitamin E. In addition to these dietary factors, other dietary constituents such as Factor 3 of Schwarz and to some extent fats rich in monoglycerides as well as some emulsifiers (for example, Tween 60) may delay the development of experimental dietary hepatic necrosis.

In contrast to cirrhosis it is difficult to reconcile pure deficiency as the possible cause of dietary hepatic necrosis with the interchangeability of substances chemically as different as the sulfur-containing amino acids, cystine or methionine, and the fat soluble vitamin E in the prevention of hepatic necrosis. The assumption has been made that the beneficial effect of the sulfur containing amino acids and tocopherol more probably results from an underlying

substances. In the case of dietary hepatic necrosis such toxic substances may originate in the intermediary metabolism or under the influence of the intestinal flora particularly in the large intestine.

Our first approach was directed toward the elimination—or at least modification—of the intestinal flora as a possible source of factors injurious to the liver. It has been shown that aureomycin, when added to the nertogenic experimental basal diet containing yeast as the sole source of protein, had a significantly beneficial effect in the prevention of experimental hepatic necrosis in rats. In contrast to vitamin E or the sulfur-containing amino acids, cystine or methionine which as supplements to the basal experimental diet will permanently prevent the production of hepatic necrosis, aureomycin was found to delay as a rule the appearance of necrosis and thus its protective action was only temporary.

Sponsored by the Commission on Liver Diseases, Armed Forces Epidemiological Board and supported in part by the Office of The Surgeon General, Department of the Army.

TABLE 1
DIETARY FACTORS IN LIVER INJURY

	Cirrhosis	Necrosis
Protein	Beneficial	Beneficial
Methionine	Beneficial	Beneficial
Cystine	Injurious	Beneficial
Choline	Beneficial	No effect or injurious
Vitamin E	No effect	Beneficial
Dietary fat	Injurious	No effect or injurious
Vitamin B ₁₂	Beneficial	No effect

In analyzing this effect of aureomycin, it should be pointed out that intensive studies have revealed no indication of an underlying systemic or focal hepatic infection as the direct cause of experimental dietary acute necrosis of the liver. Thus the choice lies among (1) the supply of a missing antinecrogenic substance, (2) a direct metabolic, and (3) an antimicrobial effect on the intestinal flora. As one possible "contaminant" we have used vitamin B₁₂ as a supplement to the basal diet, with or without added aureomycin, and found it without any appreciable effect. Although a direct metabolic effect of aureomycin could not be excluded and it still remains a possibility, we favored interaction between aureomycin and the intestinal flora as the determining factor.

If the effect of aureomycin is mediated by the suppression of the intestinal flora, other antimicrobial agents should also prove to be effective, although not necessarily equal to aureomycin depending mainly on their bacterial "spectrum" and on the ease with which they may produce resistant strains. Thus, it seemed advisable to study the effect of various antimicrobial agents on the production of dietary hepatic necrosis, especially in comparison with the effect of aureomycin.

In one of the experiments we compared aureomycin with polymyxin and streptomycin, and found polymyxin ineffective, streptomycin slightly effective and aureomycin very significantly effective. The delay in the production of massive necrosis by aureomycin becomes particularly evident when the number of surviving animals is charted in relation to days of survival.

In addition to polymyxin chloramphenicol and bacitracin were without effect on the production of dietary hepatic necrosis in rats. Sulfaguanidine had a slightly protective effective effect whereas streptomycin, neomycin, and terramycin in increasing order were definitely effective.

Ingested streptomycin is absorbed from the intestinal tract only in traces. Thus its beneficial effect further suggests suppression of the intestinal flora as the mode of action. Observations with penicillin are in good accord with this assumption. Whereas penicillin given by injection was either without any, or of only limited beneficial effect, penicillin given by mouth, especially its poorly soluble organic base salts, exerted very marked protection, not less than that of aureomycin.

With regard to this comparison between aureomycin and oral penicillin, the qualifying remark has to be made that during the course of these investigations,

TABLE 3

Treatment	Total liver fat (%)	Food intake (gm./day)
Aureomycin	25.4 ± 2.0	6.5 ± 0.2
Terramycin	12.2 ± 2.2	6.2 ± 0.2
Methionine	8.8 ± 0.7	6.7 ± 0.2
Aureomycin-Methionine	13.2 ± 2.4	6.6 ± 0.2
Terramycin-Methionine	7.5 ± 0.7	6.0 ± 0.2
	7.6 ± 0.9	6.0 ± 0.3

TABLE 4

No. of rats	Sex	Diet	Average gain	Cirrhosis
10	M	Y5H	-41.2 ± 11.0	9
10	M	Y5H + A	+16.3 ± 6.5	0
10	F	Y5H	-34.8 ± 6.4	10
10	F	Y5H + A	+19.6 ± 3.6	2

production of fatty liver and dietary cirrhosis in rats fed a cirrhosis-producing ration low in protein (casein) and high in fat. In such rats, they exerted a very pronounced hypotrophic effect. This effect may be noted on Table 3.

Aureomycin had a highly protective effect on dietary cirrhosis and its sequelae, such as ascites and renal injury. See Table 4.

Similar results were obtained with penicillin mixed with the food either in

hepatic necrosis. Note Table 6.

During the last few years, the growth promoting effect of antibiotics has been put to a successful practical test in animal husbandry and is generally

plained by the elimination of bacteria from the intestinal tract which may use up protective constituents of the food ingested and in consequence reduce their supply for the body. Such mechanism may more easily explain the effect of antibiotics on the prevention of cirrhosis and growth than on the prevention of massive necrosis which, with the interchangeability of cystine and tocopherol as protective factors makes the interaction of toxic factors perhaps as a result of 'intestinal auto-intoxication,' a reasonable possibility to be strongly considered.

Sparing of an essential dietary constituent under influence of antibiotics has

TABLE 5

THE EFFECT OF ANTIBIOTICS ON THE DEVELOPMENT OF LIVER CIRRHOSIS IN RATS FED PEANUT MEAL DIET

Group	Supplement (mgm./day)	Weight (gm.)		Cirrhosis		K. dary injury	
		Start ng	Final	0	+	0	+
1	—	154 ± 2.7	252 ± 19.1	2	8	4	6
2	Aureomycin 5	154 ± 3.0	310 ± 7.8	8	2	8	2
3	Aureomycin 25	154 ± 2.7	307 ± 6.2	10		10	
4	Terramycin 5	154 ± 2.4	305 ± 6.1	8	2	8	2
5	Terramycin 25	152 ± 2.2	327 ± 4.6	10		10	
6	Penicillin 25	153 ± 1.8	307 ± 3.3	10		10	
7	D benz Pen 5	153 ± 2.1	325 ± 2.7	10		10	
8	D benz Pen 25	153 ± 2.3	320 ± 5.8	10		10	
9	Chloromycetin 5	152 ± 2.0	307 ± 10.0	7	3	9	1
10	Chloromycetin 25	152 ± 1.9	309 ± 5.5	10		10	

(Experimental period 150 days with 10 rats in each group)

TABLE 6

	Weight gain during first four weeks gm.
Controls	4.4 ± 1.3
Aureomycin	24.0 ± 1.05
Polymyxin	12.0 ± 1.05
Streptomycin	25.5 ± 0.9

been recently demonstrated by Popper and his associates on the example of choline. Two thirds of choline ingested is excreted by a normal person in the urine as trimethylamine or its oxidized form. Pruning with aureomycin or penicillin will reduce the excretion of trimethylamine after ingestion of choline to very low levels. Intravenous injection of choline will not lead to urinary excretion of trimethylamine. These observations seem to indicate that the intestinal flora even in a normal person may convert a large proportion of ingested choline into trimethylamine. Suppression of the intestinal flora or, at least, of its choline-metabolizing constituents by aureomycin or penicillin will make more choline available for the body. By analogy, similar mechanisms may be postulated for other lipotropic food constituents, effective in the prevention of dietary cirrhosis.

Such easy solution however is not borne out by more intensive analysis of all participating factors. In the first place in collaboration with Popper and his associates, we found that the sparing of dietary choline under the influence

dietary necrotic liver degeneration is nothing unusual or special. It has been very clearly demonstrated in various laboratories that almost all vitamin B deficiencies in the rat can be inhibited or even prevented by the supplementation of various antibiotics^{1, 2, 3, 4}. Their mode of action has not been clarified. I am sure, however, that it would be unjustified to infer from such observations that all these different manifestations of avitaminosis are "gastrointestinal intoxications."

References to the Discussion

- 1
2
3
4

GERMFREE ANIMALS AND LIVER NECROSIS*

By T D Luckey, J A Reyniers, Paul Gyorgy, and M Forbes

LOBUND Institute for Research in the Life Sciences, University of Notre Dame, Notre Dame Ind., and School of Medicine, University of Pennsylvania, Philadelphia, Pa

During the latter part of 1949, discussions between the group working on the etiology of massive hemorrhagic necrosis of the liver at the University of Pennsylvania and the research staff at LOBUND Institute led to preliminary experiments early in 1950 to explore the possibility of using germfree rats in this study. It was found that the syndrome was readily produced in conventional rats from the LOBUND strain and that autoclaving the diet¹ did not materially alter its necrogenic character. Critical experimentation with germfree rats should determine whether the microbial flora affects the incidence of liver necrosis. The results obtained with germfree rats up to the present date are summarized in this report.

Methods

Germfree animals were reared and maintained in the Reyniers germfree system.² The germfree units are provided with locks in which materials such as food and water can be introduced into the cage with steam sterilization. Sterile air is obtained via glass fiber filtration. The operator cares for the animals through attached rubber gloves. The animals are housed in ordinary wire bottom rat cages or in metabolism cages when urine and fecal samples were collected. At weekly intervals, bacteriological examinations are made to establish whether the cage is germfree.³

The diet used was patterned after that of Himsworth.⁴ The composition is: corn starch 79.5 gm, British brewer's yeast, 18.0 gm, salts (USP III), 3.0 gm, peanut oil, 5.83 gm, cod liver oil, 1.17 gm, thiamin ClHCl , 4.0 mg, riboflavin 0.4 mg, pyridoxine Cl , 0.3 mg, calcium pantothenate, 2.0 mg, cobalamine, 0.02 mg, and 2 me naphthaquinone, 0.2 mg. The diet is placed in a cloth bag in an inch thick layer and treated in the sterile lock by exposure to a 25 in vacuum for 10 min then free flowing steam for 5 min followed by 25 min steam sterilization at 17 p.s.i. A vacuum is then drawn for 10 min and the food taken into the germfree cage or taken out for the conventional animals. Food and water are presented *ad libitum*. The animals given vitamin E supplements are fed about 30 mg α tocopherol per os semiweekly.

In the first two experiments, Caesarean born, hand reared germfree rats were used, in the last two experiments, dam suckled germfree animals were used. None of the differences found between germfree and conventional rats seemed to disqualify *a priori* the germfree rat as a proper test animal.

Results

The incidence of liver necrosis in the germfree and conventional rats is summarized in TABLE I.

* Sponsored by the Commission on Liver Diseases, Armed Forces Epidemiological Board and supported in part by the Office of The Surgeon General, Department of the Army and the Office of Naval Research.

TABLE 1

EFFECT OF NECROGENIC DIET ON GERMFREE AND CONVENTIONAL RATS

Status	Supply to diet	Average initial weight in gm	Growth gm/day	Days on diet at death	No. of rats dead with liver necrosis* / no started
germfree	None	84 ± 5	1.2	74 & 79	0/2
conventional	None	84 ± 5	0.7	165 ± 97	5/6†
germfree	None	40	1.4	39 (34 & 43)	0/2
conventional	None	42 ± 3	0.7	33 ± 3	8/8
germfree	None	42	1.9	69	0/2
germfree	Vit E	42	1.8	69	0/2
conventional	None	47 ± 4	0.5	41 ± 11	8/8
conventional	Vit E	54 ± 4	0.9	180	
germfree	None	39	1.7	91 & 145	0/2
germfree	Vit E	37	1.6	145	0/2
conventional	None	46 ± 5	0.8	68 + 23	12/12
conventional	Vit E	42 ± 4	1.0	150	0/4

* Incidence of necrosis based on histological examination by Doctor Harry Goldblatt

† One died with lung abscess

In the first experiment, rats with an initial weight of 85 gm were started on the necrogenic diet. The germfree rats survived for some 74 and 79 days on diet, and their livers were found to be normal. The control rats however did not come down with necrosis until much later rendering the experiment conclusive. The cause of death of the germfree animals was found to be associated with disturbances of the blood clotting mechanism as evidenced by

control animals died with liver necrosis in an average time of 32 days. The germfree animals survived for 34 and 43 days. One germfree animal had an elongated tooth and did not seem to eat well. It was found moribund on the 43rd day and the animal was killed. Necropsy revealed hemorrhagic areas in the lungs. The other animal was found to be moribund on the 43rd day. The animal was bled from the abdominal aorta, and clotting time and prothrombin time were both found to be delayed compared to that of conventional rats. The livers of both rats were found to be normal.

Since vitamin E was the only vitamin lacking which was thought to be required during this period the third experiment included two germfree rats supplemented with vitamin E and two without supplement. The eight conventional controls promptly came down with necrosis in an average of 41 days. The germfree rats ate more and grew some, but better than the conventional

TABLE 2
ALTERATIONS IN BLOOD CLOTTING AND HEMOLYSIS OF RED BLOOD CELLS IN
GERMFREE AND CONVENTIONAL RATS

Status	Supplement	Days on diet	Clotting Time (Min.)		Hemolysis after	
			Whole blood	Pro-thrombin ^a	10 hrs	20 hrs
Germfree	None	34	*			
	None	43	38	60		
	None	74	*	10		
	None	79	*			
	None	91	*			
	None	145	80	60	—	—
	Vit E	145	12	5.9		
	Vit F	145	12	6.4		
Conventional	None	27			++	++
	None	68	0.5	0.5	+	+
	None	68	0.5	0.3		
	None	70	0.5	0.3		
	Vit E	113	0.5	0.3	—	—
	Vit E	113	0.5	0.3	—	—

^a Clotting times were not taken in these animals, however extensive hemorrhages found at death in lungs and other organs. Their livers were normal.

[†] 8 rats (all other data are expressed for individual rats)

In repeating the third experiment, 12 conventional control rats died with liver necrosis in an average time of 68 days. No hemorrhages were evident in the two germfree rats supplemented with vitamin E. The two unsupplemented germfree animals lived 91 and 145 days, respectively, and had no liver necrosis. One animal died at 91 days after a blood sample was taken from the tail and on necropsy was found to have a stomach hemorrhage. The other rat showed no pathology.

The vitamin E stores of the unsupplemented germfree rats were estimated by the hemolysis test of Rowe and Gyorgy.⁶ The negative hemolysis observed for these rats (TABLE 2) would indicate that the rats were not depleted of their stores of vitamin E.

Discussion

Thus far we have been unable to induce liver necrosis in germfree rats using a diet which regularly produced lethal liver necrosis in conventional rats.

The observed disturbances in the clotting mechanism of the germfree rats were suggestive of a deficiency of vitamin K. In our experience, a vitamin K deficiency is not easily induced in germfree rats. Although 40 per cent of the vitamin K may be destroyed by autoclaving as a dietary constituent,² the

deficiency is properly
in E hemo-
tes of con-

ventional rats fed the necrogenic diet normally hemolyze after 1-6 hours exposure to dialuric acid. In simultaneous tests, erythrocytes of nonsupplemented germfree rats failed to hemolyze even after 20 hrs. Such a negative hemolysis

Summary

In a series of four experiments, eight germfree rats were found to have normal livers at autopsy. All but one of the 34 conventional rats, fed the same diet, died with massive hemorrhagic necrosis of the liver. The germfree rats exhibited prolonged clotting time which was partially prevented by vitamin E supplementation.*

Acknowledgments

This work was supported, in part, by contract with the Office of Naval Research and the Army Medical Corps. The authors acknowledge, with thanks, the help of Dr H. A. Gordon, Chief Pathologist, Morris Wagner, Chief Bacteriologist, B. A. Teah, Supervisor of Germfree Production and J. Pleasants, biochemist from LOBLUND Institute, and Dr Harry Goldblatt of the Institute for Medical Research, Cedars of Lebanon Hospital, Los Angeles.

References

1. GYORGY P. & H. GOLDBLATT. 1951. *Proc. Soc. Exptl. Biol. Med.* 76: 124.
2. REYNIER J. A., P. C. TREXLER & R. F. FRUTKIN. 1946. *LOBLUND Repts.* 1: 1.
3. REYNIER J. A., P. C. TREXLER, R. F. FRUTKIN, M. WAGNER, H. A. GORDON, T. D. LUCKY, R. A. BROWN, G. J. MANNERING & C. J. CAMPBELL. 1950. *J. Nutrition* 41: 31.
4. OLICK A. J. 1932. *J. Am. Med. Assoc.* 110: 1658.
5. ROSE C. S. & P. GYORGY. 1950. *J. Hemat.* 5: 1067.
6. HOVE E. L., H. H. COPELAND & W. D. SALMON. 1949. *J. Nutrition* 59: 397.

*Definite hemorrhagic necrosis of the liver was observed in six germfree rats since this report was given. The germfree rats reported here ate almost twice as much as conventional rats, whereas the germfree rats which developed liver necrosis were fed a diet restricted in quantity to that of the conventional control rats.

HEPATIC INJURY DUE TO CONDITIONED SULFO AMINO ACID DEFICIENCY*

By Hans Popper, J de la Hueriga, and Dieter Koch Weser

Hektoen Institut for Medical Research Cook County Hospital and the Departments of Pathology of the Cook County Hospital and Northwestern University Medical School, Chicago Ill

Functional and structural changes resulting from nutritional deficiency of amino acids and, specifically, of sulfo amino acids are well known. This paper will present examples of lesions resulting from drainage of sulfo amino acids from the body or from biological antagonism to them. Since these functional and structural changes can be prevented or corrected by additional administration of relatively large amounts of the respective amino acids, they are considered examples of conditioned amino acid deficiency.

Bromobenzene combined with cysteine is excreted in the urine after acetylation. This represents a mechanism of depletion of cysteine and its precursors such as methionine from the liver and growth inhibition after administration of bromobenzene has been described.^{1,2} If 150 mgm of bromobenzene is given intraperitoneally to a fasting rat, an extensive centrilobular necrosis develops within 48 hours. It is characterized by eosinophilic necrosis, hydropic swelling and, finally, disappearance of the liver cells associated with interstitial infiltration by inflammatory cells³ (A in FIGURE 1). The lesion is more advanced in instances in which fasting for more than 12 hours precedes the bromobenzene injection and massive necrosis with hemorrhage⁴ imitating nutritional hepatic necrosis may be noted. Administration of cysteine or methionine in large doses prevents the development of the lesion (B in FIGURE 1). This already suggests the possibility that drainage of sulfo amino acids rather than an intoxication with the halogenated benzene, causes the lesion. Chemical

— the histologic finding. In contrast to controls, the phosphatidylesterase and succinic dehydrogenase activities are markedly reduced in bromobenzene intoxicated by bromobenzene.

Summary, serum glutathione levels are low, and homocysteine retention is noted. In the animals treated simultaneously with methionine and cysteine most of these changes are either entirely prevented or at least reduced (FIGURE 2).

Additional evidence supports this concept. The mercapturic acid excretion resulting from the administration of bromobenzene is markedly increased by the simultaneous administration of cysteine and methionine resulting in prevention of the hepatic lesion (TABLE 1). The greater portion of bromobenzene is detoxified by means other than mercapturic acid excretion such as formation of glucuronates or sulfates. Nevertheless, only a small increase of the fraction of bromobenzene detoxified as mercapturic acid is associated with the protection. This suggests that the protection is not due to the removal of relatively small amounts of bromobenzene by detoxification via mercapturic acid but rather is due to repletion or sparing of the sulfo amino acids in the hepatic pool. In the case of glycine used for hippuric acid formation, such a pool of 10

* Supported by a grant from the Doctor Jerome D. Solomon Memorial Research Foundation.



FIGURE 1. Rat liver. A: X100, 48 hours after administration of 100 mg/kg of the liver effluent. B: X120, 48 hours after administration of 100 mg/kg of the liver effluent. The texture is not altered.

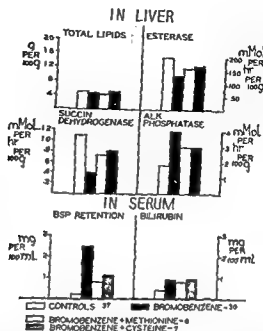


FIGURE 2 Results of chemical determinations on liver tissue and serum of control rats and rats sacrificed 48 hours after administration of 150 mgm bromobenzene/100 gm body weight with or without supplemental administration of 300 mgm methionine or cysteine/100 gm body weight. (Small figures in the legend indicate the number of animals.)

TABLE I

URINARY MERCAPTURIC ACID EXCRETION OF CONTROL RATS AND OF ANIMALS WHICH HAD RECEIVED 48 HOURS BEFORE 150 MGm BROMOBENZENE PER 100 GM BODY WEIGHT WITH OR WITHOUT ADDITIONAL ADMINISTRATION OF 300 GM METHIONINE OR 300 GM CYSTEINE/100 GM BODY WEIGHT

	Mercapturic acid	
	As mgm cysteine	As % bromobenzene injected
Controls	1	—
Bromobenzene	32	13.9
Bromobenzene + Methionine	53	23.2
Bromobenzene + Cysteine	52	23.1

mg/100 gm body weight has been demonstrated.⁵ In the case of bromobenzene, 30 mgm cysteine are made available for mercapturic acid formation.

The cysteine binding takes place on the para position of the bromobenzene molecule. Consequently, benzenes dihalogenated in the para position such as para dibromobenzene and para chlorobromobenzene do not produce the lesion. In contrast, meta dichlorobromobenzene with the para position free produces it (TABLE 2). This is confirmed by low mercapturic acid production with the para compounds and high production with the meta compound. Again, the

TABLE 2

 URINARY MERCAPTURIC ACID EXCRETION OF RATS DURING 48 HOURS FOLLOWING THE
 ADMINISTRATION OF EQUIVALENT AMOUNTS OF BROMOBENZENE OR D-HALOGENATED
 BENZENES

	Amount given body weight	As excreted	
		Amount	As given compound received
Bromobenzene	75	18	15.6
monochlorobromobenzene	91	1	16.5
p-chlorobromobenzene	91	5	4.3
p-dibromobenzene	112	4	3.5

percentage of the halogenated compound excreted as mercapturic acids rather small. These findings militate strongly against a primary toxicity of the halogenated benzenes but support drainage of sulfo amino acids as the cause for the hepatic necrosis. Finally, in animals which spontaneous liver damage occurs it is reflected in focal necroses apparently due to infection of bromobenzene injection site to produce the characteristic necrosis. If the concept presented is correct the process of detoxification as such may become lethal to the liver.

Another example of condition of amino acid deficiency in the case described above is the effect of ethionine an amino acid similar to methionine except that the terminal methyl group is replaced by an ethyl group. This supposed biological antagonist to methionine¹ has been shown to produce pancreatic necrosis followed by pancreatic fibrosis and atrophy² and lesion of the pancreatic ducts upon prolonged treatment. In late stages the acinar cells reveal an irregular regeneration. Whereas in early stages the liver appears entirely normal hydropic swelling of the glandular cells results in some of the animals treated for longer than one month (FIGURE 3). In some of these animals the urine contains reducing substances. However, the blood sugar curve is normal and further investigations are required to elucidate this glycosuria. These lesions in an organ as sensitive to protein deficiency as the pancreas are prevented or corrected by methionine administration.

Intraperitoneal administration of single doses of ethionine produces fatty liver in female rats but not in males. Females are protected by administration of testosterone and males are made sensitive by castration. This points to a protective effect of male sex hormone as far as the acute fatty liver is concerned. It can best be explained by a protein sparing effect of testosterone preventing the



FIGURE 3 ($\times 150$) Pancreas of rat which had been on 0.5 per cent eth on its diet for 51 days. There is hydropic swelling of the islet cells, distortion of the acini with marked regeneration of the acinar epithelium and dilatation of the ducts and interstitial inflammatory infiltration.

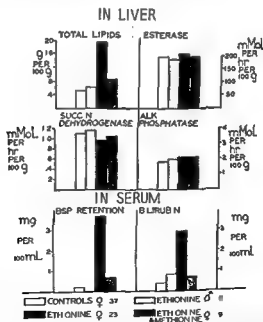


FIGURE 4 Results of chemical determinations on liver tissue and serum in control rats as well as in rats which had received 100 mg/m ethionine per 100 gm body weight 48 hours before sacrificing, with or without supplemental administration of 300 mg/m methionine per 100 gm body weight. (Small figures in the legend indicate the number of animals.)

liver develops within approximately one week. After about 12 days, fat is seen only in the central lobular zone. However, now there is central necrosis and streamers of interstitial infiltration extending into the lobular parenchyma chiefly around intralobular cholangioles (A in FIGURE 5). After three weeks extensive interstitial infiltration around the cholangioles is associated with marked liver damage and, after four weeks on the diet, a diffuse hepatic lesion is noted in male as well as in female rats¹⁶. It is characterized by diffuse hepatocellular damage with hemogenization of the cytoplasm recognized by reddish discoloration in Mallory's aniline blue or Gomori's trichrome stained section (B in FIGURE 5). This damage often progresses to cytolysis and is also associated with bizarre regeneration of the liver cells which reveal large nuclei and large and often multiple nucleoli (A in FIGURE 6). Moreover there is a dense interstitial infiltration by reticuloendothelial cells. In the center of the lobules lipofuscin deposition reflects preceding central necrosis. The fibers of the reticulum framework reveal extensive proliferation. However dissection of the lobules by septa is only occasionally seen. There is increased visibility of intralobular cholangioles, the presence of which in the normal liver was recently emphasized by Elias¹⁶. These intralobular cholangioles are not easily seen but become readily apparent if the common duct is ligated. The septal and interlobular bile ducts proliferate and the latter may reveal marked dilatation (B in FIGURE 6). This is also associated with a marked dilatation of the large extrahepatic biliary duct to about 2 mm in diameter similar to the changes recently described in mice bearing grafted pituitary tumors¹.

The characteristic lesion described is associated with reduced fat and protein content, low esterase and high phosphatase activity in hepatic tissue and increased bilirubin, markedly high alkaline phosphatase and low esterase activity in the serum. The morphologic and chemical alterations can be prevented if one per cent methionine is added to the diet or when the animals are returned for one week, or longer to the stock diet (FIGURE 7). During the course of the chronic ethionine intoxication the hepatic lipids first show a peak and subsequently a depression below the normal level whereas the proteins consistently decrease (FIGURE 8).

The characteristic diffuse hepatocellular degeneration with cholangiolitis and interstitial infiltration is subject to various influences. Only few rats survive the simultaneous administration of cortisone and ethionine, since most of them die during this period from focal hepatic necrosis apparently due to infection and not due to ethionine. However rats which survive the simultaneous administration have nearly normal livers. Administration of large doses of choline with the diet prevents the inflammatory changes but produces marked regenerative changes of the liver cells. The hepatic lesion caused by chronic ethionine feeding is not significantly altered by administration of pancreatin, vitamin B₁₂, or large amounts of fat or by cortisone. It is markedly ag-





FIGURE 6. Rat liver. A: X2.5. After 15 days of regeneration. The nuclei are large. Bile canals are present. The liver cells show cytolysis as well as irregular arrangement. There is extensive interstitial infiltration by round cells. B: X60. After 1 day on the 0.5 per cent diet. Marked dilatation of bile ducts is noted.

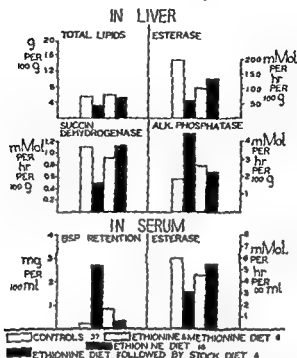


FIGURE 7. Result of chemical determination on liver and serum in control rats as well as in rats which have been on the 0.5 per cent ethionine diet for 28 days, with or without supplemental 1 per cent methionine and in rats which have been on the ethionine diet for the same period and subsequently on the stock diet for 10 days. (Small figures in the legend indicate the number of animals.)

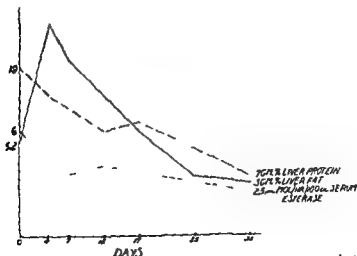
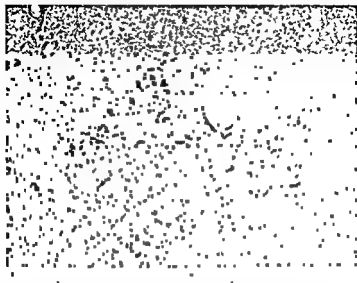


FIGURE 8. Protein and fat in hepatic tissue and esterase in serum in rats for various periods on 0.5% ethionine diet.

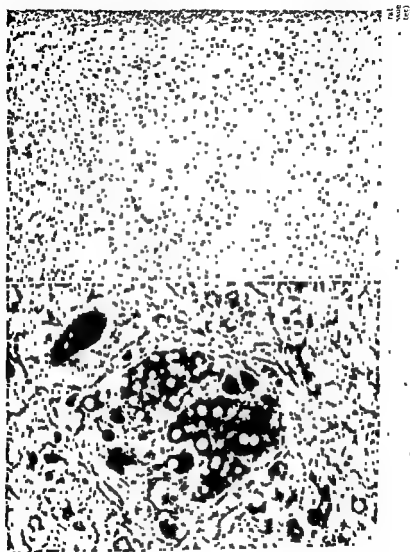


been kept for approximately 100 days on diets containing 0.2 per cent ethionine, the lesions are markedly intensified. The bile duct epithelium may reveal bizarre and excessive proliferation characterized by irregular mitoses and by

contrast to the extensive fiber proliferation between single cell plates in the surrounding liver tissue (A in FIGURE 10). These groups of cells may become

proliferations, no information is as yet available as to the biological behavior of these liver cell nodules. Nevertheless their histologic characteristics strongly suggest them to be hepatomas at least of the benign variety. Further study is required of these bile duct and liver cell nodules. Usually, more than one such lesion has been found in the 17 rats with nodules. Their response to methionine treatment particularly requires study.

Thus, the ethionine lesion imitates the picture of human malnutrition, especially kwashiorkor, since it starts with fatty metamorphosis, proceeds



rotic changes, and terminates in lesions at least suspicious for hepatic tumor
 nation, and since it is associated with pancreatic alterations. An amino acid
 agonism has to be assumed rather than a toxic effect of ethionine in produc-
 these lesions, although the latter cannot be completely excluded. Triethyl-
 re, a possible metabolite of ethionine¹⁹ does not produce hepatic and pan-
 c alterations under similar conditions
 us appears that most of the available evidence militates against a toxic
 if both bromobenzene and ethionine and suggests that depletion of, or
 ism to, sulfo amino acids is the basis of the lesions demonstrated in
 acuity and deficiency meet. The lesions represent patterns of a con-
 amino acid deficiency in a broad sense of "malnutrition caused by
 other than a primary dietary deficiency"¹⁹ It may be worth while
 for a similar pattern in man which might explain lesions which are
 nutritional deficiency but to internal interference with amino acid
 metabolism

References

1. R. W. JACKSON, 1935 The effect of bromobenzene on the utilization of methionine by the growing rat. *J Biol Chem* 111 907
2. J. B. J. A. 1940 Homocysteine in relation to the synthesis of mercapturic acids. *J Biol Chem* 133 117
3. KOCH WESER D. J. DE LA HUEGA & H. POPPER 1952 Hepatic necrosis due to bromobenzene and its dependence upon available sulfur amino acids. *Proc Soc Exptl Biol Med* 79 196
4. KOCH WESER D. J. DE LA HUEGA C. YESINCA & H. POPPER 1953 Hepatic necrosis due to bromobenzene as an example of conditioned amino acid deficiency. *Metabolism* 2 245
5. ARMSTRONG R. V. & A. NEUBURGER 1951 Hypuric acid synthesis in the rat. *Biochem J* 80 154
6. STOKOL J. A. & R. WEISS 1949 A study of the growth inhibition by diethyl and diethionine in the rat and its alleviation by the sulfur-containing amino acids and choline. *J Biol Chem* 179 1047
7. FARNER E. & H. V. SMITHSON & H. TARVER 1950 Studies on ethionine II The interference with lipid metabolism. *J Biol Chem* 182 91
8. FARNER E. & H. POPPER 1950 The production of acute pancreatitis with ethionine and its prevention by methionine. *Proc Soc Exptl Biol Med* 74 833
9. GOLDBERG R. C. & I. L. CHAILOFF 1951 Selective pancreatic acinar destruction by diethylamine. *Arch Path* 82 250
10. DE ALMEIDA, A. L. & M. L. CROSSMAN 1952 Experimental production of pancreatitis with ethionine. *Gastroenterology* 20 554
11. WACHSTEIN M. & E. MEISEL 1951 Protein depletion enhances pancreatic damage produced by ethionine. *Proc Soc Exptl Biol Med* 77 569
12. FARNER E. D. KOCH WESER H. POPPER 1951 The influence of sex and of testosterone upon fatty liver due to ethionine. *Endocrinology* 48 205
13. KOCH WESER D. E. FARNER & H. POPPER 1951 Fatty liver with and without necrosis: histological and biochemical investigations. *Arch Path* 81 498
14. POPPER, H. D. KOCH WESER & J. DE LA HUEGA 1952 Serum hepatic enzymes in experimental liver damage. *J Natl Hosp* 1 19 246
15. KOCH WESER D. & H. POPPER 1952 Hepatic fibrosis produced by chronic ethionine feed. *Proc Soc Exptl Biol Med* 79 34
16. ELIAS H. 1952 Morphology of the liver. *Trans 11th Conf. Josiah Macy Jr Found*
17. FURTH J. F. L. GABRY & A. C. USTOV 1952 Hyperplasia and cystic dilatation of the extrahepatic biliary tracts in mice bearing grafted pituitary growth. *Cancer Research* 12 739
18. McARTHUR C. S. & C. C. LEWIS 1950 Oral toxicity and hepatotropic potency of triethyl homologue of choline. *Biochem J* 46 226
19. EASTOFF B. H. 1948 Conditioning factors in nutritional disease. *Physiol Revs* 28 107

CLINICAL EVALUATION OF A HIGH PROTEIN HIGH CARBOHYDRATE RESTRICTED FAT DIET IN THE TREATMENT OF VIRAL HEPATITIS*†

By N C Leone Frank Ratner William C L Diefenbach Miriam G Eads
Jacob E Lieberman and Roderick Murray

Laboratory of Biologics Control National Microbiological Institute National Institutes of Health
Bethesda Md

Introduction

The relationship between diet and certain types of liver injury has been established in the case of animals^{1 2 3 4 5} and specific liver protective factors have been described^{4 6 7}. Results of such studies however, do not appear to be directly applicable to viral hepatitis in man because this disease has not been transmitted successfully to animals. The clinical importance of diet in the treatment of hepatitis in man has often been emphasized but the degree of this has not been entirely clear. The quantity of fat in the diet has been

what amounts to a clinical trial involving a number of variables. The prolonged nature of such studies in hepatitis the lack of early specific diagnoses in many situations¹⁸ and the absence of established uniform methods of evaluation.

The present study was directed towards the evaluation of the effect of a high

Epidemiological Board and with the cooperation of the Bureau of Virus U S Department of Justice. The findings reported here are the product of study of 67 controlled cases of induced homologous serum hepatitis which occurred among volunteers in two institutions.

Conduct of Study

The volunteer participants were men between the ages of 21 and 35. In the latter part of the study men of 30 years and over were not accepted. Final selection was based upon a personal interview a medical history a physical examination and a review of the available records to determine the suitability

of the volunteers on the basis of age, physical condition mental status and personality. In addition complete series of liver function tests were run including serum bilirubin (one minute and total), thymol turbidity, cephalin flocculation, thymol flocculation, total lipids, zinc turbidity, and urine urobilinogen and bilirubin.

The nature of the over all project made it possible to observe the course of the disease from the earliest appearance of abnormal laboratory or clinical findings. It was possible, therefore, to study the effects of diet starting at a much earlier period in the illness than could normally be possible in the case of casual hospital patients. Every effort was made to take advantage of this feature of the study by placing patients on a dietary regimen as early as possible in the course of their illness. Good control was achieved and the design was such as to provide for the collection of data in terms of well defined periods in the patient's course from pre inoculation to discharge.

Throughout the entire study, two diets applicable or adaptable for clinical use were employed. As the subjects manifested signs or symptoms of hepatitis, they were admitted to the hospital and placed on a high protein high carbohydrate, restricted fat diet, referred to here as the special diet or on an *ad lib* diet.

maximum voluntary food consumption.

The *'ad lib'* diet consisted of the same food provided for the general institution population. There were no restrictions made on the type character quantity or quality of food other than the general limitations of the institution diet.

Subjects remained on the respective diets from the time of admission until

TABLE 1

Period	Special diet				Ad lib diet				Difference			
	Cal	Protein	Fat	CHO	Cal	Protein	Fat	CHO	Cal	Protein	Fat	CHO
Acute	3616	203.3	78.8	541.5	2213	77.5	78.2	30.3	1403	125.8	0.6	254.2
Recovery	3754	221.0	80.1	599.1	2833	102.1	95.0	392.0	1121.0	118.4	18.1	206.9
Total	3844	215.2	92.5	604.4	2609	93.8	90.5	361.7	1235.0	121.4	14.5	217.7

TABLE 2
DIETARY VALUES IN GRAMS

Sampling period (days)*	Diet	By calculation		By chemical analysis	
		Protein	Fat	Protein	Fat
7	Special	210 5	86 3	192 3	68 6
7	Ad lib	111 3	110 7	94 9	96 9
7	Special	209 3	87 8	198 1	76 7
7	Ad lib	101 6	100 9	92 9	77 6
7	Special	215 0	97 0	193 0	100 7
7	Ad lib	106 5	106 9	100 5	76 0
7	Special	203 7	93 9	212 1	80 2
7	Ad lib	100 2	110 1	92 8	76 3
7	Special	254 9	116 1	219 2	117 0
7	Ad lib	100 8	107 1	94 3	81 5
7	Special	241 4	122 1	209 6	97 7
7	Ad lib	105 4	119 3	97 5	87 0
7	Special	221 3	84 3	221 8	90 6
7	Ad lib	113 3	106 0	91 6	95 9
7	Special	208 0	93 1	190 9	67 9
7	Ad lib	127 6	138 8	96 2	96 8
5	Special	171 8	68 3	229 0	91 1
5	Ad lib	104 3	101 3	96 7	75 2
5	Special	190 5	70 9	226 6	85 3
5	Ad lib	118 3	96 9	135 1	73 9
1	Special	253 3	61 0	288 6	105 2
1	Ad lib	116 8	104 8	104 6	78 0
2	Special	229 8	110 2	223 2	103 7
2	Ad lib	99 2	95 9	86 3	81 3
2	Special	221 3	72 0	311 1	61 7
2	Ad lib	133 9	141 2	139 8	103 0

* Complete meals were homogenized in a blender. Aliquots of each meal were pooled for the period indicated and samples of these pools set aside for chemical analysis.

under supervision. Edible waste or uneaten food was also weighed and the amount recorded. Food intake figures were calculated for each patient using these data. Sample meals representative of both diets were chemically analyzed for comparison with the calculated values.*

Sixty seven volunteers who developed hepatitis with jaundice were included in the present study. Of these, 32 were in the "special" diet group and 35 in the "ad lib" diet group. During the time this study was being conducted, a total of 95 individuals was started on one or the other of the dietary regimens.

A number of these patients were already in the hospital with hepatitis when the dietary study was commenced. Since only those cases in which it was possible to calculate dietary intakes for the complete period of hospitalization were considered for inclusion in the group, eleven of these initial subjects were excluded, seven on the "special" diet and four on the "ad lib" diet. Two further cases, both on the "special" diet, were excluded because of uncertainty as to their dietary intake,* as were five individuals for whom a diagnosis of hepatitis with jaundice could not be sustained. All of these 18 individuals were eliminated before the statistical analysis was made. Initially, the distribution of patients between the two diet groups was on a one-for-one basis, a separate series of alternations being followed in each of the two institutions. Due to the imbalance of the numbers between the two diet groups occurring as a result of the elimination of the above 18 individuals (five on the "ad lib" diet and 13 on the "special" diet), the last ten patients admitted to the dietary study were distributed on a two-for-one basis.

The cases of hepatitis studied resulted from parenteral inoculation of the following infecting materials

A Infected pool plasma A single lot of infected plasma was used in all the work relating to the safety of blood products. This pool was prepared from commercially procured plasma to which had been added some 800 ml of plasma containing the "Fort Bragg" strain of homologous serum hepatitis virus as well as a total of approximately 600 ml of plasma or serum from four reputed cases of serum hepatitis. The original volume of the material was approximately 130 liters, and it is referred to here as "infected pool plasma." The subjects who received this material represent a homogeneous group.

B Infected pool plasma, treated This represents materials which remained infective after treatment by one of the following processes

- (1) Ultraviolet irradiation at various levels
- (2) Heating for 2 hours at 60° C
- (3) Heating for 4 hours at 60° C
- (4) Dilution to 10⁻¹ and 10⁻²
- (5) Treatment of β propiolactone at different levels or
- (6) Storage at room temperature for 3-6 months

Subjects who received these materials represent a heterogeneous group in the sense that, although they all received infected pool plasma, this had been treated in a variety of ways each presumably affecting the virus in different degree.

C Carrier material Six suspected carriers of homologous serum hepatitis were examined by inoculation of their plasma into volunteer subjects. Five of the six were proved to be carriers. In addition, a few cases resulting from first passage infected pool material have been placed in this group.

Cases resulting from the inoculation of some of the "carrier materials" had short incubation periods in the range 10 to 70 days, whereas those who received infected pool plasma had incubation periods between 60 and 120 days. Thus, difference in incubation periods coupled with a somewhat different cl

* One of these subjects received infected pool plasma; the other infected pool plasma treated during which their total serum γ globulin levels remained above 2.0 mgm per 100 ml were 63 days respectively.

picture in which the severity of symptoms at the onset of illness was greater in relation to the laboratory findings than would be expected on the basis of our experience with the other infecting materials studied, suggests that some of the cases receiving "carrier material" may have had infectious hepatitis induced by parenteral inoculation.

D Thrombin Thrombin from a commercial batch reported to have caused cases of hepatitis in clinical use was inoculated into ten subjects in order to determine the safety of thrombin with respect to homologous serum hepatitis. Four of the subjects who developed hepatitis were studied from the dietary point of view. They do not fall into any of the above groups, so it is difficult to evaluate them except from the over all point of view as to the development of complications.

The distribution of the 67 cases between the two diet groups, broken down by

for the study

(2) The incubation period, beginning with inoculation and extending to the time at which a diagnosis of hepatitis could be made

(3) The period of illness which was divided into the acute period and the recovery period

These periods were defined roughly by changes in liver function tests and, therefore, permitted objective comparisons

Cases of all degrees of severity were encountered including three which ended fatally. These latter cases had been respectively inoculated with infected pool plasma, infected pool plasma, treated, and carrier material.

TABLE 3A
DURATION OF ILLNESS IN DAYS—TSB 10 TO 10

Infected pool plasma		Infected pool plasma treated		Carrier material		Thrombin	
Spec	Ad lib	Spec	Ad lib	Spec	Ad lib	Spec	Ad lib
79	78	36	70	63	46	22	25
62	86	49	56	81	49		13
85	86	63	130	92	35		27
98	102	42	84	21	20		
93	70	56	49	30	43		
*	47	28	56	74	24		
102	45	53	47	*	47		
105	45	15	46	56			
94	63	45	18				
	50	*	70				
		15	67				
		35	21				
		14	42				
		98	22				
			59				
Total No. Cases	9	14	15	11	7	1	3

* Fatal cases (not included in the analysis)

The statistical evaluation of the effect of the two diets was made in terms of the duration of illness of the 67 cases. It should be noted that in such a comparison when recovery is the end point it is not feasible to include cases which do not recover. For this reason the three fatal cases are excluded from this analysis although it is obvious that they must be taken into account when evaluating the results.

Discussion of Statistical Methods

Most of the analysis was based on measures of duration such as the number of days during which liver function tests particularly the total serum bilirubin (TSB), remained abnormal or the number of days of clinical illness *etc*. The data have been examined in a number of ways to try and discern differences between the cases on the "special" diet and those on the ad lib diet and

TABLE 3b
DURATION OF ILLNESS—TSB 10 TO 10

Diet	Infected pool plasma	Infected pool plasma treated	Control	Thrombosis	Sum
Special Σ	718	549	417	22	1706
Σx_i	89 7500	42 2308	59 5714	22 0000	58 8276
n_i	8	13	7	1	29
Ad lib Σ	672	837	272	65	1846
Σx_i	67 2000	55 8000	38 8071	21 6667	52 7429
n_i	10	15	7	3	35
Sum Σ	1390	1386	689	87	3532
ΣN	77 2222	49 5000	49 2143	21 7000	50 5000
$d = \Sigma x_i - \Sigma x_i =$	18	28	14	4	64
$n_i = \frac{n_i}{N} =$	22 5500	-13 5692	20 7143	0 3313	15 6587
$n_i d_i =$	4 4444	6 9643	3 5000	0 7300	78 5658
$n_i d_i^2 =$	100 2212	-94 5000	72 5946	0 2000	5048 0672
	2259 9881	1281 2894	1405 7064	0 0833	

$$(\Sigma n_i d_i^2) / \Sigma n_i = 394.1952$$

ANALYSIS OF VARIANCE

Source	Sum of squares	df	Mean square	Mean square	df	Sum of squares	Source
Diet ignoring material Interaction Material	587	1					
	4654	3	1551.29	1551.29	3	14607	Material ignoring diet Interaction Diet
	14414	3	4804.67	394	1	4654	
Between cells Within cells	19655	56					
	27453	3					
Total Mean	47110	63					
	197136	1					

$$\text{Interaction } F = \frac{1551.29}{490.27} = 3.16 \quad F_{0.05} = 2.8$$

TABLE 3c
DURATION OF ILLNESS—TSB 10 TO 10

Source	SS	df	MS	
Infected Pool Plasma				
Mean	107339	1		
Total	7397	17		
Diet	2260	1	2260	$F = \frac{2260}{321} = 7.04 \quad F_{.05} = 4.49$
Within diet	5136	16	321	
Infected pool plasma, treated				
Mean	68607	1		
Total	18789	27		
Diet	1282	1	1282	$F = \frac{1282}{673} = 1.90 \quad F_{.05} = 4.22$
Within diet	17507	26	673	
Carrier				
Mean	33909	1		
Total	6189	13		
Diet	1501	1	1501	$F = \frac{1501}{391} = 3.84 \quad F_{.05} = 4.75$
Within diet	4697	12	391	

TABLE 3d
DURATION OF ILLNESS—TSB 10 TO 10

Source	SS	df	MS	
Special Diet				
Mean	100360	1		
Total	24558	28		
Internal	12590	3	4197	$F = 8.76 \quad F_{.05} = 2.99$
Residual	11968	25	479	
Ad lib diet				
Mean	97363	1		
Total	21965	34		
Internal	6477	3	2159	$F = 4.32 \quad F_{.05} = 2.91$
Residual	15488	31	500	

When differences existed, to determine their statistical significance. A "five per cent" level of significance was decided upon before any calculations were undertaken. This implies that a difference, measured in terms of its standard error, could have occurred by chance only once in 20 times.

Tables 3A, 3B, 3C, and 3d illustrate the analysis in the case of the duration of illness as judged by the time the total serum bilirubin remained above 1.0 mgm per 100 ml.

Three fundamentally separate tests have been employed in this analysis: the tests for duration, body weight change, and the incubation period.

It was apparent early in the analysis that the variation in the measures selected for study was influenced by the type of infecting material as well as by diet. The analysis of the data relating to the period before diets were started brings out a number of differences associated with the type of infecting material. One such characteristic is the incubation period. The average incubation periods were 103 days, and carrier material, 61 days. The average incubation period of the differences between infecting materials which is free of diet effect is body weight loss during the incubation period. The respective average differences are infected pool plasma, ~ 5.4 lbs, infected pool plasma, treated, ~ 5.5 lbs, and carrier material, ~ 3.6 lbs.

The weight loss data are based on fewer than the 67 patients, because either the inoculation or hospitalization weight was not obtained in a few instances. However, if the assumption is accepted that a missed weight could occur with the same probability in the group receiving different infecting materials, then the available numbers are sufficient to establish the difference between infecting materials.

The data on incubation periods and on loss of body weight occurring during the incubation period can be used to test whether bias was introduced in the assignment of patients to the diets. If bias existed, it might result in a preponderance of patients with short incubation periods being assigned to one of the diet groups. No such difference was found. Similarly, there was no difference between diets as measured by weight loss during the incubation period.

Results

A Food consumption levels TABLE 1 summarizes the calculated food intake levels in terms of average consumption during the acute period, the recovery period, and for the total period of illness. It will be seen that differences of well over 1,000 calories, 100 gm in the case of protein and over 200 gm in the case of carbohydrate, were maintained between the two diets.

B Chemical analysis of diets Chemical analyses of the diets were made to confirm calculated values. These are compared in TABLE 2.

C Effect of diet In the course of the analysis, significant interaction between diet and the different infecting materials was found. That is, the effect of diet was not consistent with respect to infecting material. Where this interaction appeared in the analysis, it was necessary to analyze separately the effect of diet within each group which received different infecting material. The following measures of effect were used:

- The number of days the TSB remained abnormal when 1.0 was taken as the endpoint (duration of time the TSB was elevated above 1.0 mgm per 100 ml).
- The number of days during which the bilirubin remained abnormal when 1.5 mgm per 100 ml was taken as the endpoint. As a corollary to this, the period of time taken for the TSB to fall from 1.5 to 1.0 was studied.
- The number of days for the TSB to reach its maximum level.
- The number of days for the TSB to return from maximum to 1.0 mgm per 100 ml.

(e) The number of days during which the patient was ill, $\pm e$, from the time of onset of illness until the patient could be said to be improving clinically

(f) The duration of the recovery period, $\pm e$, from time of the onset of clinical recovery until the bilirubin reached 1.0

(g) Weight change during illness

(h) Average maximum TSB levels in each of the diet groups This measure of effect can also be used in part as a test for bias

The above measures of effect have been analyzed statistically It must be emphasized that (a) through (g) are interrelated and do not constitute separate analyses They represent measures of the duration of illness or portions of it as judged by different end points

The results are summarized in TABLE 4 They reveal the following

(1) With return to a bilirubin level of 1.0 mgm per 100 ml taken as the

TABLE 4
EFFECT OF DIET

Measure of effect	Infecting material	Average		Remarks
		Special diet	Ad lib diet	
Duration of illness (TSB 1.0 to 1.0)	IPP* IPP, treated 'Carrier'	Days†		Significant diet effect No significant diet effect No significant diet effect
		90	67	
		42	56	
		60	39	
Duration of illness (TSB 1.0 to 1.5)	IPP IPP, treated 'Carrier'	72	52	No significant diet effect
		24	36	No significant diet effect
		45	23	Significant diet effect
Duration from TSB 1.5 to 1.0	All groups	17	16	No significant diet effect
Duration from TSB 1.0 to max.	All groups	20	16	No significant diet effect
Duration from max TSB to 1.0	All groups	39	37	No significant diet effect
Acute period	All groups	14	17	No significant diet effect
Recovery period	IPP IPP treated 'Carrier'	55	47	No significant diet effect
		25	35	No significant diet effect
		41	21	No significant diet effect
Incubation period	All groups	85	88	No significant difference between diet groups
Maximum TSB	All groups	mgm/100 ml		No significant difference between diet groups
		11.0	9.7	
Wt change between inoculation & TSB 1.5	IPP IPP, treated 'Carrier'	lbs		Significant diet effect Significant diet effect No significant diet effect
		1.4	-8.6	
		-2.1	-6.3	
		-1.0	1.1	

* Infected pool plasma

† To nearest whole day

endpoint of recovery

(a) Subjects who received infected pool plasma and were given the "special" diet had a course of illness which was on the average 23 days longer than the corresponding individuals on the "ad lib" diet This difference was statistically significant

(b) Subjects who received infected pool plasma, treated, and were given the "special" diet had a course of illness which was on the average 14 days shorter than corresponding individuals on the "ad lib" diet This difference did not reach statistical significance

(c) Subjects who received "carrier material" and were given the "special" diet had a course of illness which was on the average 21 days longer than corresponding individuals on the "ad lib" diet This difference was not statistically significant.

(2) With return to a bilirubin level of 1.5 mgm per 100 ml taken as the point of recovery, results were similar and in the same direction in each of the categories listed in 1(a), (b) and (c) above The differences however reached statistical significance only in the case of "carrier material" When the period between the 1.5 TSB and 1.0 TSB levels was studied, no statistically significant effect of diet could be demonstrated.

(3) In calculating the average duration of time between a bilirubin level of 1.0 and the maximum TSB no statistically significant diet effect was demonstrable

(4) In calculating the average duration of time between the maximum TSB and the endpoint of recovery at 1.0 no significant diet effect was demonstrable

(5) In calculating the average duration of the acute period no statistically significant diet effects were apparent Because of interaction however it was necessary to make separate calculations with respect to infecting materials when examining the average recovery periods Here the differences were analogous to those obtained in 1(a), (b) and (c) However the differences were not statistically significant

(6) There were no significant diet effects on weight change during the period from hospitalization to the fall of TSB to 1.5

D Effect of infecting materials The statistical evaluation disclosed a marked effect due to the type of infecting material used Whenever interaction was present it was necessary to carry out the analysis with respect to each of the measures of effect within each diet group separately These results are summarized in TABLE 5 Reference to this table discloses the following

(1) Within each diet group, there was a significant effect due to the infecting material used whether the endpoint of the duration of illness was measured by the return of the TSB level to 1.5 or to 1.0

(2) There was a significant effect of infecting material on the period between a TSB level of 1.5 and a TSB level of 1.0

(3) There was no significant effect of infecting material on the average number of days from 1.0 to the maximum TSB level There was however a significant effect on the average number of days from the maximum TSB level to

TABLE 5
EFFECT OF INFECTING MATERIAL

Measure of effect	Diet	Average			Remarks
		Infected pool plasma	Infected pool plasma treated	Carrier	
		Days*			
Duration of illness (TSB 1 0 to 1 0)	'Special' "Ad lib"	90 67	42 56	60 39	Significant effect Significant effect
Duration of illness (TSB 1 0 to 1 5)	'Special' "Ad lib"	72 52	24 36	45 23	Significant effect Significant effect
Duration from TSB 1 5 to 1 0	Both	16	19	15	Significant effect
Duration from TSB 1 0 to max	Both	24	15	17	No significant effect
Duration from max TSB to 1 0	Both	53	34	33	Significant effect
Acute period	Both	17 5	12	18	No significant effect
Recovery period	'Special' "Ad lib"	55 5 47	25 35	41 21	Significant effect Significant effect
Incubation period	Both	81	103	61	Significant effect
Maximum TSB	Both	mgm /100 ml			Significant effect
		15 4	8 0	10 1	
		lbs			No significant effect Significant effect
% change between Inoc & TSB 1 5	"Special" "Ad lib"	1 4 -8 6	-2 1 -6 3	-1 0 1 1	

* To nearest whole day

0 The same effects were apparent when the period of illness was broken down into acute and recovery phases

(4) There was a significant effect of infecting material on the average maximum TSB levels

(5) There were significant differences between the mean incubation periods of the infecting materials used

(6) There was no significant effect on weight changes of infecting material during the period between hospitalization and fall of TSB to 1 5

E Relation between diet and the development of complications All cases on the dietary study were evaluated with respect to development of the following complications: death, coma, pre coma, recrudescence, relapse, prolonged illness (more than 4 months), and residua persisting beyond the time of discharge from the program. The latter included persistent hepatomegaly, splenomegaly, right upper quadrant pain or tenderness, or failure of hepatic tests to return to pre inoculation levels. Since these complications cannot be given equal

TABLE 6

Definition	Special no	Diet %*	Ad lib no	Diet %*
•	4	12.5	0	0
•	5	15.6	1	2.9
•	13	40.6	5	14.3
•	15	46.9	6	17.1
•	15	46.9	7	20.0
•	18	56.2	8	22.8

All but one of the above definitions (B) yield statistically significant differences indicating a higher occurrence of complications among those who received the special diet.

* In terms of the total number cases in each diet group.

weight, a number of definitions employing increasingly restrictive criteria were applied. The definitions and the number of cases falling into each are shown in TABLE 6.

The definitions listed are to some extent arbitrary and as they are not independent it must not be assumed that the data have been subjected to an

diet

In compiling this table cases were counted only once. Cases with more than one of the listed complications were counted only under the most restricted definition. Two of the cases listed under definition A require special mention. These subjects were members of a group of five volunteers who had been inoculated with plasma obtained from a proved carrier who had developed hepatitis with jaundice some 6 months before the plasma was collected. These two cases developed fulminating hepatitis with incubation periods of 44 and 46 days. One of the cases terminated fatally. The remaining three subjects in the group did not develop hepatitis. The unusual severity of the illness produced by this particular inoculum suggests, though this cannot be proved, that the outcome was related more to the infecting material than to the diet. Even if the two cases are removed from the table however, the over-all results are not appreciably changed.

Discussion

All of the cases of hepatitis included in the present study were of so-called 'inoculation hepatitis'. Most were undoubtedly serum hepatitis, but there is some reason for believing that the illness resulting from the inoculation of some of the 'carrier materials' may have been infectious hepatitis. Because of this the broader term 'viral hepatitis' has been used in the title.

The present cases developed in the course of a study on the sterilization of blood and blood products. This study was limited in scope and there was no way of knowing beforehand how many cases of hepatitis could be anticipated. Consequently no effort was made to randomize patients with respect to both

diet and infecting material. A simple one-for-one allocation of patients between the diet groups was followed, in the hope that a practical randomization would thereby occur. A study of the incubation periods of patients with different inoculating materials (a measure free of the effect of infected different inoculating materials) was indeed achieved.

The special feature of the present study is the fact that it was possible to place patients on one or other of the dietary regimens at the earliest appearance of signs, symptoms, or laboratory findings suggestive of hepatitis. This institution of dietary therapy must be taken into consideration in any interpretation of the results of the present study.

The severity and duration of illness in viral hepatitis are extremely variable. In the present study the analysis was based only on objective findings and the duration of elevation of the total serum bilirubin, and it should be emphasized that although different endpoints and subdivisions of this measure of effect were used in the analysis, these are not independent but are in parts of the same analysis.

Summary

- (1) A group of 67 volunteers taking part in studies on viral hepatitis been evaluated with respect to two diets: a high protein, high-carbohydrate restricted fat diet and an ad lib diet.
- (2) It was possible to institute the diet in each case at an early point in measures of duration studied.
- (3) The type of infecting material had a significant effect on most of measures of duration studied.
- (4) Apparent differences in effect of the two diets did not reach the level of significance with most of the measures of duration studied. In those instances where this did occur the average duration of illness of patients on the "ad lib" diet was shorter.
- (5) The occurrence of complications was more frequent among patients receiving the high protein, high-carbohydrate, restricted fat diet.

References

1. BEST, C. H., J. M. HERSHEY, & M. E. HIRTSMAN. 1932. The control of the deposition of liver fat. *Am. J. Physiol.* 101: 7.
2. WEICHELBAUM, T. E. 1935. Cystine deficiency in the albino rat. *Quart. J. Exptl. Med.* 70: 185.
3. GYÖRGY, P., & H. GOLDBLATT. 1939. Hepatic injury on a nutritional basis in nine of hemorrhage and necrosis of the liver of rats. *Proc. Soc. Exptl. Biol. Med.* 33: 109.
4. DART, F. S., W. H. SEBRELL, & K. D. LILLIE. 1942. Prevention of cystine or methionine deficiency in the albino rat. *Proc. Soc. Exptl. Biol. Med.* 33: 109.
5. SCHWARTZ, K. 1944. Tocopherol als Leberschutzstoff. *Z. physiol. Chem.* 231: 109.
6. SCHWARTZ, K. 1951. A hitherto unrecognized factor against dietary necrosis of degeneration in American yeast (Factor 3). *Proc. Soc. Exptl. Biol. Med.* 78: 8.
7. GYÖRGY, P., J. STOKES, JR., W. H. SMITH, & H. GOLDBLATT. 1950. Studies on the effect of aureomycin in hepatic disease. II. The effect of aureomycin on the treatment of acute hepatitis. *Brit. Med. J.* 1: 111.
8. GERTZEN, O. 1945. The Medical Department Dietetic Service in the Medical U. S. Army, 1942-1945. *4: 24*.

